U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB80-133226

'In Vitro' Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides

SRI International, Menlo Park, CA

Prepared for

Health Effects Research Lab, Research Triangle Park, NC

Oct 79

United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Research Triangle Park NC 27711

EPA-600/1-79-041
October 1979

PB80-133226

Research and Development

EPA

In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ENVIRONMENTAL HEALTH EFFECTS RESEARCH series. This series describes projects and studies relating to the tolerances of man for unhealthful substances or conditions. This work is generally assessed from a medical viewpoint, including physiological or psychological studies. In addition to toxicology and other medical specialities, study areas include biomedical instrumentation and health research techniques utilizing animals — but always with intended application to human health measures.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/1-79-041	2.	3. RECIPIENT'S ACCESSION NO. PB80-133226
4. TITLE AND SUBTITLE In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides		5. REPORT DATE October 1979
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Vincent F. Simmon, Ph.D., SRI International Menlo Park CA 94025		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Microbial Genetics Program SRI International 333 Ravenswood Avenue, Menlo Park CA 94025		10. PROGRAM ELEMENT NO. 1EA615
		11. CONTRACT/GRANT NO. 68-01-2458
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory RTP, NC Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED Final Report
		14. SPONSORING AGENCY CODE EPA 600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Eighteen pesticides being reviewed as a part of the EPA Substitute Chemical Program were tested for mutagenic activity by the following *in vitro* procedures:

- (1) Reverse mutation in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 and in *Escherichia coli* WP2 *uvrA*.
- (2) Induction of mitotic recombination in the yeast *Saccharomyces cerevisiae* D3.
- (3) Relative toxicity assays in DNA repair-proficient and -deficient strains of *E. coli* (strains W3110 and p3478, respectively) and of *Bacillus subtilis* (strains H17 and M45 respectively).
- (4) Unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).

Nine of the 18 pesticides were mutagenic in one or more of the assays. One compound, demeton, was mutagenic in all of them. Trichlorofon was mutagenic in all the assays except those for relative toxicity. Acephat was mutagenic in the *Salmonella typhimurium* in TA100, *Saccharomyces cerevisiae* D3, and UDS assays. Dicamba, 2,4-D acid, 2,4-DB acid, and propanil were positive only in the assay for relative toxicity. Disulfoton was positive only in the UDS assay, and then only in the absence of the metabolic activation system. Crotoxyphos was positive only in the *S. cerevisiae* D3 assay.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
In Vitro analysis Microbiology Mutagens Deoxyribonucleic acids Pesticides		06C,F,M,T

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 177
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE A09A01

EPA-600/1-79-041
October 1979

IN VITRO MICROBIOLOGICAL MUTAGENICITY AND UNSCHEDULED DNA SYNTHESIS STUDIES OF EIGHTEEN PESTICIDES

by

Vincent F. Simmon, Ph.D.
SRI International
Menlo Park, California 94025

Contract No. 68-01-2458

Project Officer

Dr. Michael D. Waters
Genetic Toxicology Division
Health Effects Research Laboratory
Research Triangle Park, North Carolina 27711

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The Federal Insecticide, Fungicide, and Rodenticide Act designates the Environmental Protection Agency as the governmental body responsible for the safety of all pesticides used in the United States. More recently, the Federal Environmental Pesticide Control Act (PL 92-516) strengthened EPA's regulatory responsibilities in the area of pesticides to include intra- as well as interstate commerce.

To be federally registered, a pesticide must have been determined not to be hazardous to health or to the environment when used according to its labeling restrictions. Thus, relative to new law as well as to specific directives included in Public Law 93-135, 1973, EPA now is conducting a thorough review of the implications of using alternative chemicals, including older registered pesticides, for pest control.

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

Eighteen pesticides being reviewed as part of the EPA Substitute Chemical Program were tested for mutagenic activity by the following *in vitro* procedures:

- Reverse mutation in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 and in *Escherichia coli* WP2 *uvrA*.
- Induction of mitotic recombination in the yeast *Saccharomyces cerevisiae* D3.
- Relative toxicity assays in DNA repair-proficient and -deficient strains of *E. coli* (strains W3110 and p3478, respectively) and of *Bacillus subtilis* (strains H17 and M45, respectively).
- Unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).

Nine of the 18 pesticides were mutagenic in one or more of the assays. One compound, demeton, was mutagenic in all of them. Trichlorofon was mutagenic in all the assays except those for relative toxicity. Acephat was mutagenic in the *Salmonella typhimurium* in TA100, *Saccharomyces cerevisiae* D3, and UDS assays. Dicamba, 2,4-D acid, 2,4-DB acid, and propanil were positive only in the assay for relative toxicity. Disulfoton was positive only in the UDS assay, and then only in the absence of the metabolic activation system. Crotoxyphos was positive only in the *S. cerevisiae* D3 assay.

CONTENTS

LIST OF ILLUSTRATIONS v

LIST OF TABLES vii

SUMMARY 1

INTRODUCTION 3

METHODS 5

  Microbiological Assays 5

    Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100 5

    Escherichia coli WP2 7

    Saccharomyces cerevisiae D3 8

    Escherichia coli W3110/p3478 and Bacillus subtilis H17/M45 9

    Aroclor 1254-Stimulated Metabolic Activation System 10

  Unscheduled DNA Synthesis Assay 11

    Cell Culture 12

    Dilution of Compounds 13

    Metabolic Activation 13

    Controls 13

    Test Procedure 13

    Interpretation of Results 15

RESULTS AND DISCUSSION 17

  Microbiological Assays 17

  UDS Assay 24

CONCLUSIONS 37

REFERENCES 163

ILLUSTRATIONS

1 Assays of Acephate With *Salmonella typhimurium* Strain TA100 19

2 Assays of Demeton With *Salmonella typhimurium* Strains TA1535 and TA100 20

3 Assays of Trichlorfon With *Salmonella typhimurium* Strain TA100 21

4 Assays of Demeton With *Escherichia coli* Strain WP2 22

5 Assays of Trichlorfon With *Escherichia coli* Strain WP2 23

6 Assays of Demeton With *Saccharomyces cerecisiae* D3 . . . 25

7 Assays of Crotoxyphos With *Saccharomyces cerevisiae* D3 26

8 Assay of Acephate With *Saccharomyces cerevisiae* D3 Without Metabolic Activation 27

9 Assays of Trichlorfon With *Saccharomyces cerevisiae* D3 28

10 Unscheduled DNA Synthesis Assays of Demeton 30

11 Unscheduled DNA Synthesis Assays of Acephate Without Metabolic Activation 31

12 Unscheduled DNA Synthesis Assays of Disulfoton Without Metabolic Activation 33

13 Unscheduled DNA Synthesis Assays of Trichlorfon Without Metabolic Activation 34

28 Ethion, Experiment 1 65
29 Ethion, Experiment 2 66
30 Ethion, Experiment 3 67
31 Fensulfothion, Experiment 1 68
32 Fensulfothion, Experiment 2 69
33 Fonofos, Experiment 1 70
34 Fonofos, Experiment 2 71
35 Methoxychlor, Experiment 1 72
36 Methoxychlor, Experiment 2 73
37 Propanil, Experiment 1 74
38 Propanil, Experiment 2 75
39 Propanil, Experiment 3 76
40 Siduron, Experiment 1 77
41 Siduron, Experiment 2 78
42 Trichlorfon, Experiment 1 79
43 Trichlorfon, Experiment 2 80
44 Trichlorfon, Experiment 3 81

In Vitro Assays with *Escherichia coli* WP2:

45 Acephate, Aspon, Carbofuran, Crotoxyphos, Demeton, Diazinon, Dicamba, Disulfoton, and Ethion 82
46 Acephate 84
47 Fensulfothion, Fonofos, Methoxychlor, Propanil, Siduron, Trichlorfon, 2,4-D Acid, 2,4-DB Acid, and Endrin 85
48 Trichlorfon 87
49 Differential Toxicity of Repair-Proficient and -Deficient Microorganisms 88

In Vitro Assays with *Saccharomyces cerevisiae* D3

50 Acephate 91
51 Aspon 92
52 Carbofuran 93
53 Crotoxyphos 94
54 2,4-D Acid 95
55 2,4-DB Acid 96

TABLES

1 Eighteen Pesticides Evaluated by SRI International for Mutagenicity 38

2 *In Vitro* Mutagenesis: Summary Data for EPA Pesticides 39

In Vitro Assays with *Salmonella typhimurium*:

3 Acephate, Experiment 1 40

4 Acephate, Experiment 2 41

5 Acephate, Experiments 3 and 4 42

6 Aspon, Experiment 1 43

7 Aspon, Experiment 2 44

8 Carbofuran, Experiment 1 45

9 Carbofuran, Experiment 2 46

10 Crotoxyphos, Experiment 1 47

11 Crotoxyphos, Experiment 2 48

12 2,4-D Acid, Experiment 1 49

13 2,4-D Acid, Experiment 2 50

14 2,4-DB Acid, Experiment 1 51

15 2,4-DB Acid, Experiment 2 52

16 Demeton, Experiment 1 53

17 Demeton, Experiment 2 54

18 Diazinon, Experiment 1 55

19 Diazinon, Experiment 2 56

20 Dicamba, Experiment 1 57

21 Dicamba, Experiment 2 58

22 Dicamba, Experiment 3 59

23 Disulfoton, Experiment 1 60

24 Disulfoton, Experiment 2 61

25 Disulfoton, Experiment 3 62

26 Endrin, Experiment 1 63

27 Endrin, Experiment 2 64

88 Third Assay of Dicamba with Metabolic Activation 132
89 Assay of Fensulfothion 133
90 Repeat Assay of Fensulfothion 134
91 Assay of Fensulfothion with Metabolic Activation 135
92 Repeat Assay of Fensulfothion with Metabolic Activation 136
93 Assay of Endrin 137
94 Assay of Endrin with Metabolic Activation 138
95 Repeat Assay of Endrin with Metabolic Activation 139
96 Third Assay of Endrin with Metabolic Activation 140
97 Assay of Aspon 141
98 Assay of Aspon with Metabolic Activation 142
99 Assay of Carbofuran 143
100 Assay of Carbofuran with Metabolic Activation 144
101 Assay of Crotoxyphos 145
102 Assay of Crotoxyphos with Metabolic Activation 146
103 Assay of 2,4-D Acid 147
104 Assay of 2,4-D Acid with Metabolic Activation 148
105 Assay of 2,4-DB Acid 149
106 Assay of 2,4-DB Acid with Metabolic Activation 150
107 Assay of Diazinon 151
108 Assay of Diazinon with Metabolic Activation 152
109 Assay of Fonofos 153
110 Assay of Fonofos with Metabolic Activation 154
111 Assay of Ethion 155
112 Assay of Ethion with Metabolic Activation 156
113 Assay of Methoxychlor 157
114 Assay of Methoxychlor with Metabolic Activation 158
115 Assay of Siduron 159
116 Assay of Siduron with Metabolic Activation 160
117 Assay of Propanil 161
118 Assay of Propanil with Metabolic Activation 162

56 Demeton . . . 97
57 Diazinon . . . 99
58 Dicamba . . . 101
59 Disulfoton . . . 102
60 Endrin . . . 104
61 Ethion . . . 105
62 Fensulfothion . . . 106
63 Fonofos . . . 107
64 Methoxychlor . . . 108
65 Propanil . . . 109
66 Siduron . . . 110
67 Trichlorfon . . . 111

Unscheduled DNA Synthesis:

68 Assay of Demeton . . . 112
69 Repeat Assay of Demeton . . . 113
70 Assay of Demeton with Metabolic Activation . . . 114
71 Repeat Assay of Demeton with Metabolic Activation . . . 115
72 Assay of Acephate . . . 116
73 Repeat Assay of Acephate . . . 117
74 Assay of Acephate with Metabolic Activation . . . 118
75 Repeat Assay of Acephate with Metabolic Activation . . . 119
76 Assay of Disulfoton . . . 120
77 Repeat Assay of Disulfoton . . . 121
78 Assay of Disulfoton with Metabolic Activation . . . 122
79 Repeat Assay of Disulfoton with Metabolic Activation . . . 123
80 Assay of Trichlorfon . . . 124
81 Repeat Assay of Trichlorfon . . . 125
82 Assay of Trichlorfon with Metabolic Activation . . . 126
83 Repeat Assay of Trichlorfon with Metabolic Activation . . . 127
94 Assay of Dicamba . . . 128
85 Repeat Assay of Dicamba . . . 129
86 Assay of Dicamba with Metabolic Activation . . . 130
87 Repeat Assay of Dicamba with Metabolic Activation . . . 131

SUMMARY

Eighteen pesticides being reviewed as a part of the EPA Substitute Chemical Program were tested for mutagenic activity by the following *in vitro* procedures:

- Reverse mutation in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 and in *Escherichia coli* WP2 *uvrA*.
- Induction of mitotic recombination in the yeast *Saccharomyces cerevisiae* D3.
- Relative toxicity assays in DNA repair-proficient and -deficient strains of *E. coli* (strains W3110 and p3478, respectively) and of *Bacillus subtilis* (strains H17 ans M45 respectively).
- Unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).

Nine of the 18 pesticides were mutagenic in one or more of the assays. One compound, demeton, was mutagenic in all of them. Trichlorofon was mutagenic in all the assays except those for relative toxicity. Acephate was mutagenic in the *Salmonella typhimurium* in TA100, *Saccharomyces cerevisiae* D3, and UDS assays. Dicamba, 2,4-D acid, 2,4-DB acid, and propanil were positive only in the assay for relative toxicity. Disulfoton was positive only in the UDS assay, and then only in the absence of the metabolic activation system. Crotoxyphos was positive only in the *S. cerevisiae* D3 assay.

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act designates the Environmental Protection Agency as the governmental body responsible for the safety of all pesticides used in the United States. More recently, the Federal Environmental Pesticide Control Act (PL 92-516) strengthened EPA's regulatory responsibilities in the area of pesticides to include intra- as well as interstate commerce.

To be federally registered, a pesticide must have been determined not to be hazardous to health or to the environment when used according to its labeling restrictions. Thus, relative to new law as well as to specific directives included in Public Law 93-135, 1973, EPA now is conducting a thorough review of the implications of using alternative chemicals, including older registered pesticides, for pest control.

In the pesticide review process, EPA emphasizes development of scientific criteria for evaluating the safety of compounds substituted for those pesticides found to be hazardous. In addition to reviewing and evaluating the literature on pesticides and maintaining liaison with industry and academia, the strategy program includes laboratory studies to obtain additional data. One of these laboratory programs is directed toward gathering mutagenesis data on a selected number of compounds.

EPA's program is responsive to one of the recommendations included in the President's Scientific Advisory Committee Report of September 1973, Chemicals and Health. In that document, the Committee recommended that "Regulatory agencies should take steps to insure that new scientific data raising the possibility of new or extended hazards from chemicals in use are subject to careful process of scientific review for merit interpretation."

Development of methods for evaluating the mutagenic hazard of chemical compounds has advanced markedly in the last few years. In

Preceding page blank

contrast to the undefined empirical tests used a short time ago, procedures now available can detect chromosome breaks, DNA damage, and mutational events caused by chemical stress. Mutant strains of microorganisms and human fibroblast cells are effective _in vitro_ systems for reliable detection of genotoxic agents.

Many pesticide chemicals in commercial use today have not been adequately investigated for their potential mutagenic hazard. With the public's increasing concern about possible pollution of our environment by chemicals, the widely used pesticides must be evaluated.

Under contract to EPA, SRI studied 18 pesticides to determine their potential for mutagenic activitiy. We used _in vitro_ test methods that are appropriate for such evaluations. The 18 pesticides tested are listed in Table 1; the common name, trade name, manufacturer, purity, batch or lot number, and supplier are shown. (SRI had previously reported on "_In Vivo_ and _In Vitro_ Studies of Selected Pesticides To Evaluate Their Potential as Chemical Mutagens" in February 1977.[1] That study of 20 pesticides was a part of this contract, No. 68-01-2458.)

The six _in vitro_ assay systems used were the reverse mutation in _Salmonella typhimurium_ strains TA1535, TA1537, TA1538, TA98, and TA100 and in _Escherichia coli_ WP2; induction of mitotic recombination in the yeast _Saccharomyces cerevisiae_ D3; relative toxicity assays in DNA repair-proficient and -deficient strains of _E. coli_ (strains W3110 and p3478, respectively) and of _Bacillus subtilis_ (strains H17 and M45, respectively); and unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).

METHODS

Microbiological Assays

The in vitro microbiological assay systems used to examine the 18 pesticides for mutagenicity were Salmonella typhimurium (TA1535, TA1537, TA1538, TA98, and TA100), Escherichia coli WP2, repair-deficient and -proficient strains of Bacillus subtilis (H17 and M45) and of E. coli (W3110 and p3478), and the yeast Saccharomyces cerevisiae D3. In each procedure except the relative toxicity assays, an Aroclor 1254-stimulated, rat liver homogenate metabolic activation system was included to provide metabolic steps that the microorganisms either are incapable of conducting or do not carry out under the assay conditions.

The assay procedure with S. typhimurium has proven to be 80 to 90% accurate in detecting carcinogens as mutagens, and it has about the same accuracy in identifying chemicals that are not carcinogenic.[2,3] The assay procedure with S. cerevisiae is about 55% accurate in detecting carcinogens as agents that increase mitotic recombination.[4] E. coli WP2 and the relative toxicity assays are three additional methods of detecting mutagens; however, the reliability of these test methods has not been adequately validated yet. The combination of these five assay procedures significantly enhances the probability of detecting potentially hazardous chemicals.

Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100

The Salmonella typhimurium strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on a minimal media petri plate containing a trace of histidine, only those cells that revert to histidine independence (his^+) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The his^+ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar, the mutation frequency is increased 2- to 100-fold, usually in a dose-related manner.

We obtained our *S. typhimurium* strains from Dr. Bruce Ames of the University of California at Berkeley.[2,5-10] In addition to having mutations in the histidine operon, all the indicator strains have a mutation (*rfa*) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (*bio*) and in the repair of ultraviolet (uv)-induced DNA damage (*uvrB*). The *rfa* mutation makes the strains more permeable to many large aromatic molecules, thereby increasing the mutagenic effect of these molecules. The *uvrB* mutation causes decreased repair of some types of chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to *his*$^+$ by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the resistance transfer factor, plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens.[7] In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cells. We have shown that TA100 can detect mutagens such as benzyl chloride and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF2) that are not detected by TA1535. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICR-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz(a)anthracene]. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. Strain TA98 is derived from TA1538 by the addition of plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All indicator strains are kept at 4° C on minimal agar plates, supplemented with an excess of biotin and histidine. The plates with the plasmid-carrying strains contain, in addition, ampicillin (25 μg/ml) to ensure stable maintenance of plasmid pKM101. New stock culture plates are made every four to six weeks from single colony reisolates that have been checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37° C in nutrient broth (Oxoid, CM67). After stationary overnight growth, the cultures are shaken for 3 to 4 hours to ensure optimal growth.

To a sterile 13 x 100 mm test tube placed in a 43° C heating block, we add in the following order:

(1) 2.00 ml of 0.6% agar*
(2) 0.05 ml of indicator organisms
(3) 0.50 ml of metabolic activation mixture (optional)†
(4) 0.05 ml of a solution of the pesticide dissolved in DMSO.

For negative controls, we use steps (1), (2), and (3) (optional) and 0.05 ml of the solvent used for the test chemical. For positive controls, we test each culture by specific mutagens known to revert each strain, using steps (1), (2), (3) (optional), and (4).

This mixture is stirred gently and then poured onto minimal agar plates.‡ After the top agar has set, the plates are incubated at 37° C for 2 days. The number of his^+ revertant colonies is counted and recorded.

A positive response in the Salmonella/microsome assay is indicated by a reproducible, dose-related increase in the number of revertants in one or more of the tester strains.

Escherichia coli WP2

The E. coli WP2 (uvrA) strain used at SRI was obtained originally from Dr. D. McCalla.[11] It is a tryptophan auxotroph (trp) by virtue of a

---

[*] 0.6% agar contains 0.05 mM histidine, 0.05 mM biotin, and 0.6% NaCl.

[†] See page 9.

[‡] Minimal agar plates consist of, per liter, 15 g of agar, 20 g of glucose, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid monohydrate, 10 g of K_2HPO_4, and 3.5 g of $NaHNH_4PO_4 \cdot 4H_2O$.

base-pair substitution mutation in the tryptophan operon. In addition, WP2 is deficient in the repair of some physically or chemically induced DNA damage (uvrA).[12] This uvrA mutation makes the strain more sensitive to certain mutagens.

A procedure similar to the Ames Salmonella assay is used to measure the reversion of WP2 to tryptophan independence. However, the minimal agar is supplemented with 1.25 g of Oxoid nutrient broth (CM67) per liter to provide each plate with the trace of tryptophan required for enhancement of any mutagenic effect of the test chemical.[12] No additional tryptophan is added to the top agar.

Saccharomyces cerevisiae D3

The yeast S. cerevisiae D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway.[13] When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event may be increased by incubating the organisms with various mutagens. The degree of mutagenicity of a compound or of its metabolite is determined from the number of red-pigmented colonies appearing on the plates.[14]

The S. cerevisiae tester strain is stored at -80° C. For each experiment, the tester strain is inoculated in 1% tryptone and 0.5% yeast extract and grown overnight at 30° C with aeration.

The in vitro yeast mitotic recombination assay in suspension is conducted as follows. The overnight culture is centrifuged, and the cells are resuspended at a concentration of ~10^8 cells/ml in a 67 mM phosphate buffer (pH 7.4). To a sterile test tube are added:

- 1.30 ml of the resuspended culture
- 0.50 ml of either the metabolic activation mixture or buffer
- 0.20 ml of a solution of pesticide dissolved in DMSO or 0.20 ml of DMSO alone.

Several doses of the pesticide (up to 5%, w/v or v/v) are tested in each experiment, and appropriate controls are included.

The suspension mixture is incubated at 30° C for 4 hours on a roller drum. The sample is diluted serially in sterile physiological saline, and 0.2-ml aliquots of the 10^{-5} and 10^{-3} dilutions are spread on tryptone-yeast agar plates; five plates are used for the 10^{-3} dilution and three plates are used for the 10^{-5} dilution. The plates are incubated for 2 days at 30° C, followed by 2 days at 4° C to enhance the development of the red pigment indicative of adenine-deficient homozygosity. Plates of the 10^{-3} dilution are scanned with a dissecting microscope at 10X magnification, and the number of red colonies or red sectors (mitotic recombinants) is recorded. The surviving fraction of organisms is determined from the number of colonies appearing on the plates of the 10^{-5} dilution. The number of mitotic recombinants is calculated per 10^5 survivors.

A positive response in this assay is indicated by a dose-related increase in the absolute number of mitotic recombinants per milliliter as well as in the relative number of mitotic recombinants per 10^5 survivors.

Escherichia coli W3110/p3478 and Bacillus subtilis H17/M45

The E. coli strains W3110 and p3478 that are used at SRI were obtained from Dr. H. Rosenkranz, who devised the DNA polymerase repair assay.[15] Strain p3478 is a DNA polymerase-deficient (polA$^-$) derivative of W3110 and is very sensitive to the effects of some physical and chemical agents that react with cellular DNA. The repair assay is based on the finding that when exposed to agents that alter the DNA, bacteria tend to protect themselves by removing the altered DNA segment and then by resynthesizing the correct DNA sequence. Thus, their survival is enhanced. The enzyme DNA polymerase is involved in this resynthesizing process.[16] The extent of chemically induced DNA damage can be measured by comparing the relative toxicity (zone of growth inhibition) of the two strains. Therefore, if a chemical interacts with DNA, strain p3478 should be more sensitive than strain W3110 to any toxic effect due to this interaction.

The B. subtilis strains H17 and M45 were obtained from Dr. T. Kada.[17] Strain M45 ($\underline{rec}^-$) is derived from H17 but is deficient in the genetic recombination mechanism necessary to repair DNA damage. Cells deficient in this repair mechanism are killed more easily by chemical mutagens than are wild-type cells ($\underline{rec}^+$). If the chemical is toxic to $\underline{rec}^-$ cells but at the same concentration is not toxic to $\underline{rec}^+$ cells, the chemical is assumed to interact with DNA.

For each experiment, an inoculum from frozen stock cultures is grown overnight at 37° C with shaking in nutrient broth consisting of 1% tryptone and 0.5% yeast extract. A 0.1-ml aliquot of this bacterial culture (approximately 3×10^8 cells) is added to 2 ml of nutrient broth containing 0.6% agar. The suspension is mixed and poured onto the surface of a plate containing the same ingredients as the broth plus 2% agar (25 ml). When the top agar has solidified, a sterile filter disc impregnated with the test substance is placed in the center of the plate. The plates are incubated at 37° C for 16 hours; then the width (diameter) of the zone of inhibition of growth is measured. Several concentrations of the substance are usually tested. We routinely use DMSO as diluent and as solvent for crystalline chemicals.

The positive control for this assay is 1-phenyl-3,3-dimethyltriazine. The negative control is chloramphenicol, which should cause equal zones of inhibition in both strains because it is toxic to bacteria but does not kill by interacting with DNA.

Aroclor 1254-Stimulated Metabolic Activation

Some carcinogenic chemical (e.g., of the aromatic amino type or polycyclic hydrocarbon type) are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens.[8-10] Some of these intermediate metabolites are very potent mutagens in the S. typhimurium test. Ames has described the liver metabolic activation system that we use.[10] In brief, adult male Sprague-Dawley rats (250 to 300 g) are given a single 500-mg/kg intraperitoneal injection of a polychlorinated biphenyl, Aroclor 1254. This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection, the animals' food is removed, but drinking water is provided ad libitum. On the fifth day, the rats are killed and the liver homogenate is prepared as follows.

The livers are removed aseptically and placed in a preweighed sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed in an equal volume of cold, sterile 0.15 M KCl (1 ml/g of wet organ), minced with sterile surgical scissors in three volumes of 0.15 M KCl, and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 x g, and the supernatant, referred to as the S-9 fraction, is quickly frozen in dry ice and stored at -80° C.

The metabolic activation mixture consists of, for 10 ml:

- 1.00 ml of freshly thawed S-9 fraction
- 0.20 ml of $MgCl_2$ (0.4 M) and KCl (1.65 M)
- 0.05 ml of glucose-6-phosphate (1 M)
- 0.40 ml of NADP (0.1 M)
- 5.00 ml of sodium phosphate (0.2 M, pH 7.4)
- 3.35 ml of H_2O.

Unscheduled DNA Synthesis Assays

Many mutagenic and carcinogenic agents have been shown to induce unscheduled DNA synthesis (UDS) in an in vitro tissue culture system of mammalian cells.[18] UDS is a form of mammalian repair synthesis that involves at least two processes: first, the agent interacts with DNA, resulting in damage to the DNA; then follows incorporation of nucleotide(s) to repair the DNA. UDS, which occurs in a wide variety of mammalian cell types, is considered to be a fairly universal system because it has been observed in all stages of the cell cycle (G_0, G_1, G_2, and M) other than S, the normal synthetic phase.[19,20] (UDS is not observed during S-phase because the high level of incorporation of nucleotides during scheduled DNA synthesis obscures the relatively low level of incorporation of nucleotides during UDS.)

A number of chemicals have been shown to be ineffective in producing DNA damage in in vitro cultures of mammalian cells; yet in the metabolically active environment of the whole animal they are rapidly converted to mutagenic and/or carcinogenic intermediates. Therefore, the investigator must attempt to recreate this metabolic environment in vitro. This is usually done by adding a microsomal preparation from a mammalian liver homogneate to the test system. Thus, we routinely perform a parallel series of UDS assays in the presence and absence of a metabolically active environment to predict the ability of an agent to induce genetic damage.

We used the UDS assay system in the previous testing of 20 substitute pesticides.[1] Reported here are the results of UDS testing, with and without metabolic activation, of the 18 additional substitute pesticides.

Cell Culture

WI-38 cells grown in T-25 tissue culture flasks were used for the UDS assays. Replicate cultures of these cells were initiated in Eagle's Basal Medium containing 10% (v/v) fetal calf serum. The cells were grown to confluency and were maintained in medium containing 0.5% serum for 5 to 6 days preceding the UDS assays.* This produced contact-inhibited cells in synchronous cultures in the G_0 phase of the mitotic cycle. To further reduce the possibility of incorporation of ^{3}H-TdR by an occasional S-phase cell that might escape the contactinhibition synchrony and thus obscure measurements of UDS, the cultures were preincubated for 1 hour with 10^{-2} M hydroxyurea (HU) before each assay and 10^{-2} M HU was added during each subsequent step of the assays.

---

* As a check against the presence of mycoplasma, which could incorporate tritiated thymidine (^{3}H-TdR) and thus obscure measurments of UDS, stock cultures were periodically sent to Microbiological Associates, who cultured them on Difco Beef Heart Infusion agar or broth for analysis for the presence of mycoplasma. The results of these analyses were consistently negative.

Dilution of Compounds

Immediately prior to each assay, the pesticide was diluted in an appropriate solvent (ethanol or DMSO) to form a series of concentrations that, when diluted into culture medium, yielded the appropriate set of test concentrations. To facilitate solubilization or achieve an even suspension of the stock solutions of the compounds in solvent, some of the compounds were sonicated for a brief period of time prior to dilution. The final concentration of solvent was maintained at 1% or less, which we have previously found to be not cytotoxic.

Metabolic Activation

For testing with metabolic activation, a preparation consisting of the 9000 x *g* supernatant of a liver homogenate (250 mg of liver/ml) from adult Swiss-Webster mice was used. To this was added the following cofactors: nicotinamide, 3.05 mg/ml; glucose-6-phosphate, 16.1 mg/ml; $MgCl_2 \cdot 6H_2O$, 5.08 mg/ml; and NADP, 0.765 mg/ml.

Controls

The positive controls were 4-nitroquinoline-N-oxide (4NQO), a compound that induces UDS in the absence of a metabolic activation system, and dimethylnitrosamine (DMN), a compound that induces UDS *in vitro* only when an exogenous metabolic activation system is incorporated into the treatment protocol. The negative control was the solvent diluted in culture medium.

Test Procedure

The contact-inhibited WI-38 cells were incubated at 37° C with dilutions of the pesticides and with 1 μCi/ml of ^{3}H-TdR (specific activity, 6.7 Ci/mmole). For testing in the absence of metabolic activation, the cells were exposed simultaneously to the pesticide and to ^{3}H-TdR for 3 hours. For testing with metabolic activation, the cells were incubated together with pesticide, ^{3}H-TdR, and the metabolic activation preparation for 1 hour. (The shorter exposure time for metabolic activation testing was used because longer exposures of WI-38 cells to the liver homogenate preparation could be cytotoxic.) In both cases, the cells were then incubated with ^{3}H-TdR and HU, but without pesticide, for an additional 3 hours.

DNA was extracted from the cells using a modification of the PCA-hydrolysis procedure;[21] one aliquot of the DNA solution was used to measure the DNA content, after the reaction with diphenylamine,[22] and a second aliquot was used for scintillation-counting measurements of the extent of incorporation of ^{3}H-TdR. The results were expressed as disintegrations per minute (dpm) of incorporated ^{3}H-TdR per unit of DNA and were compared with the rate of incorporation of ^{3}H-TdR into cells exposed to solvent only (negative controls).

We have defined as an acceptable assay one in which the response of the positive control compound is predicted, within the 95% confidence limits, by regressions of average dpm/μg DNA versus average dpm/μg for background.[23] The regressions that follow are based on data that we have acquired in previous testing:

Type of Testing	Regression*	Sample Size (n)	Correlation Coefficient (r)
Without metabolic activation	$Y_1 = 629 + 16.42\ (X)$†	55	0.8066
With metabolic activation	$Y_2 = 212 + 2.11\ (X)$†	25	0.8307

If the observed average level of incorporation for the positive control compound is outside the 95% confidence limits of the regression, we assume that some variation has occurred in the experimental procedures and the test is repeated.

---

* Regressions over a range of background dpm/μg DNA of 0 to 450.

† Y_1 = Average dpm/μg DNA for 10^{-5} M 4NQO (positive control).
Y_2 = Average dpm/μg DNA for 5×10^{-2} M DMN (positive control).
X = Average dpm/μg DNA for background (negative control).

Interpretation of Results

We have tested 41 compounds of defined carcinogenic activity, based on the results of *in vivo* bioassays, and have analyzed these results using either the parametric One-Way Classification Analysis of Variance or the nonparametric Kruskal-Wallis One-Way Analysis of Variance, depending on which was more appropriate.* Of the 16 compounds generally recognized as being direct-acting carcinogens, 15 induced statistically significant elevations in the incorporation of ^{3}H-TdR into DNA, at the 99% confidence level. In all but three of these, the response was dose-related. The assay of the sixteenth carcinogen, *p*-rosaniline, failed to suggest a positive response. Of the 13 compounds reported to be noncarcinogenic, only one (glycidol) had a statistically significant positive response, which was not dose-related. Thus, it appears that the 99% confidence limits of these statistical analysis coupled with the indication of a dose-response relationship can be used with reasonable accuracy to predict the biological significance of the UDS response to an ultimate carcinogen or a noncarcinogen.

The correlation between UDS response and biological significance for testing with metabolic activation is less clear. Of the 12 procarcinogens (compounds requiring chemical modifications to become active) that we have tested with metabolic activation, seven induced statistically significant increases in ^{3}H-TdR uptake at the 99% confidence level, all of which were dose-related. The remaining 5 procarcinogens failed to indicate any increase in ^{3}H-TdR incorporation. Thus, it appears that the metabolic activation preparation presently used for UDS testing is capable of activating only a portion of the spectrum of procarcinogens. However, for those that can be activated, a statistically significant and dose-related response can be observed. Thus, if these two criteria are met, we can interpret the results as indicating the repair of DNA damage by the cells in response to the compound being evaluated. However, lack of a positive response in testing cannot be assumed to be indicative of an absence of potential biological hazard.

---

* If there is reason to believe that the variances of each of the treatments in a test are equal (i.e., Bartlett's test of the variance is negative), the parametric analysis is the appropriate one. If the variances are not equal, the nonparametric analysis is the appropriate one.[24,25]

RESULTS AND DISCUSSION

The results of the in vitro microbiological and UDS assays are summarized in Table 2. A positive response in these assays is defined as a reproducible, dose-related increase in the effect being observed. A genotoxic or mutagenic effect was observed for 9 of the 18 pesticides tested. The 9 pesticides that had a positive response on one or more of the assays were acephate, crotoxyphos, 2,4-D acid, 2,4-DB acid, demeton, dicamba, disulfoton, propanil, and trichlorfon. Demeton was positive in all the assays. Trichlorfon was positive in all but the relative toxicity assays with *E. coli* and *B. subtilis*. Acephate was weakly mutagenic in *S. typhimurium* TA100, increased mitotic recombination in *S. cerevisiae* D3, and increased unscheduled DNA synthesis in WI-38 cells. No toxicity was observed in the relative toxicity assays for either acephate or trichlorfon; therefore, the negative result in these assays may indicate that the pesticide did not diffuse into the agar. Dicamba, 2,4-D acid, 2,4-DB acid, and propanil were positive in the relative toxicity assays but were without activity in all the other assays. Crotoxyphos increased mitotic recombination in *S. cerevisiae* D3 and disulfoton increased UDS, but no other effects were observed for these two pesticides. Except for the relative toxicity assays, dose-response curves are presented for pesticides that gave a positive response. Aspon, carbofuran, diazinon, endrin, ethion, fensulfothion, fonofos, methoxychlor, and siduron were not genotoxic or mutagenic in any of the six assays we performed.

Microbiological Assays

Each pesticide was tested at least twice on separate days, using one plate per dose. The first experiment was a test over a wide range of doses to look for toxicity or mutagenicity. If no toxicity or mutagenicity was observed, the second experiment was conducted at higher concentrations. If mutagenicity was observed, a dose response was determined. An assay that gave a mutagenic response was always repeated to confirm that the results were reproducible.

Tables 3 through 44 present the results of the microbiological assays in agar with Salmonella typhimurium. In this reverse-mutation assay system, three pesticides were mutagenic. Acephate (Tables 3-5) was very weakly mutagenic in assays with strain TA100 at doses above 2500 μg/plate. Although the response was marginal (the greatest increase observed was 61 revertants above a background of 150 spontaneous revertants in Experiment 3), Figure 1 shows that it was reproducible and dose-related. Demeton (Tables 16 and 17) was strongly mutagenic in strains TA1535 and TA100, inducing as much as a 50-fold increase in revertants above the background in strain TA1535 and a 10-fold increase in TA100; Figure 2 presents the dose-response curves. Demeton was slightly more mutagenic when the metabolic activation mixture was added to the plate than when it was not.

Trichlorfon increased reverse mutations in strain TA100 only (Tables 42-44; Figure 3). However, it was only moderately mutagenic, with a 3- to 5-fold increase in revertants above the background frequency. It was more mutagenic without the addition of the metabolic activation mixture (Experiment 3). In previous studies, trichlorfon was not found to be mutagenic in the dominant lethal[26] and Drosophila[27] assays.

We observed slight increases in the number of revertants with strain TA100 in the assays with ethion (Tables 28-30). However, because the amount of this pesticide was limited, we were unable to test at doses greater than 5 mg/plate. We believe additional tests using higher doses are needed to determine whether a dose response could be obtained.

Tables 45 through 48 present the results of the assays with E. coli WP2. Demeton and trichlorfon were mutagenic in this reverse-mutation assay. Figures 4 and 5 show the dose response. Their order of mutagenic activity was the same as in the S. typhimurium assays; i.e., demeton was more mutagenic than trichlorfon.

Table 49 presents the results of assays for microbial inhibition in repair-deficient and -proficient strains of B. subtilis and E. coli.

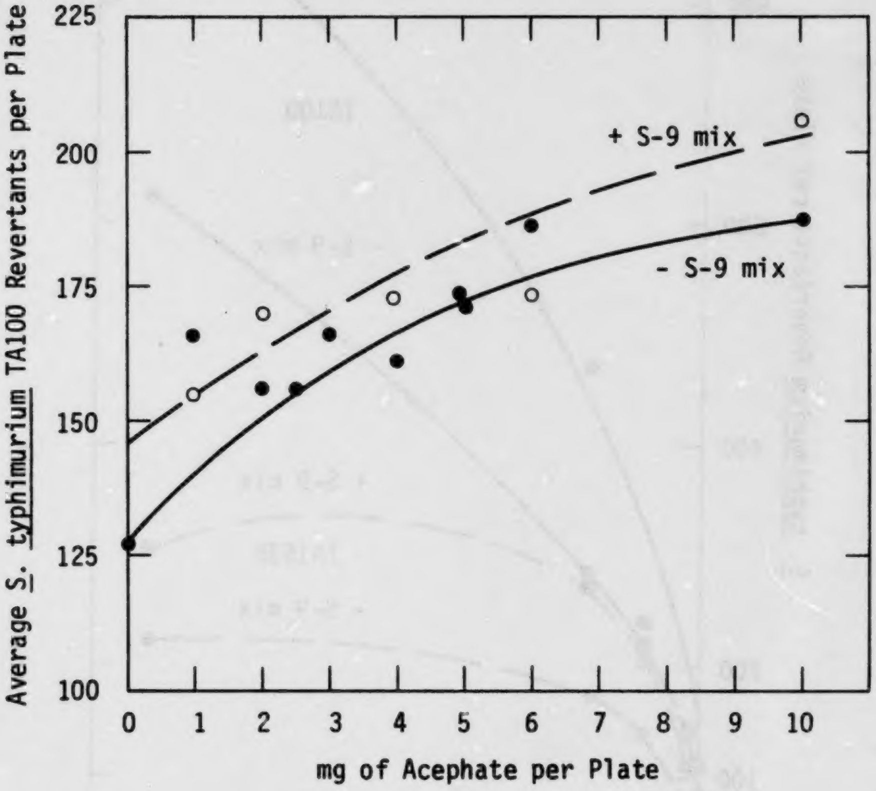


FIGURE 1 ASSAYS OF ACEPHATE WITH SALMONELLA TYPHIMURIUM STRAIN TA100.

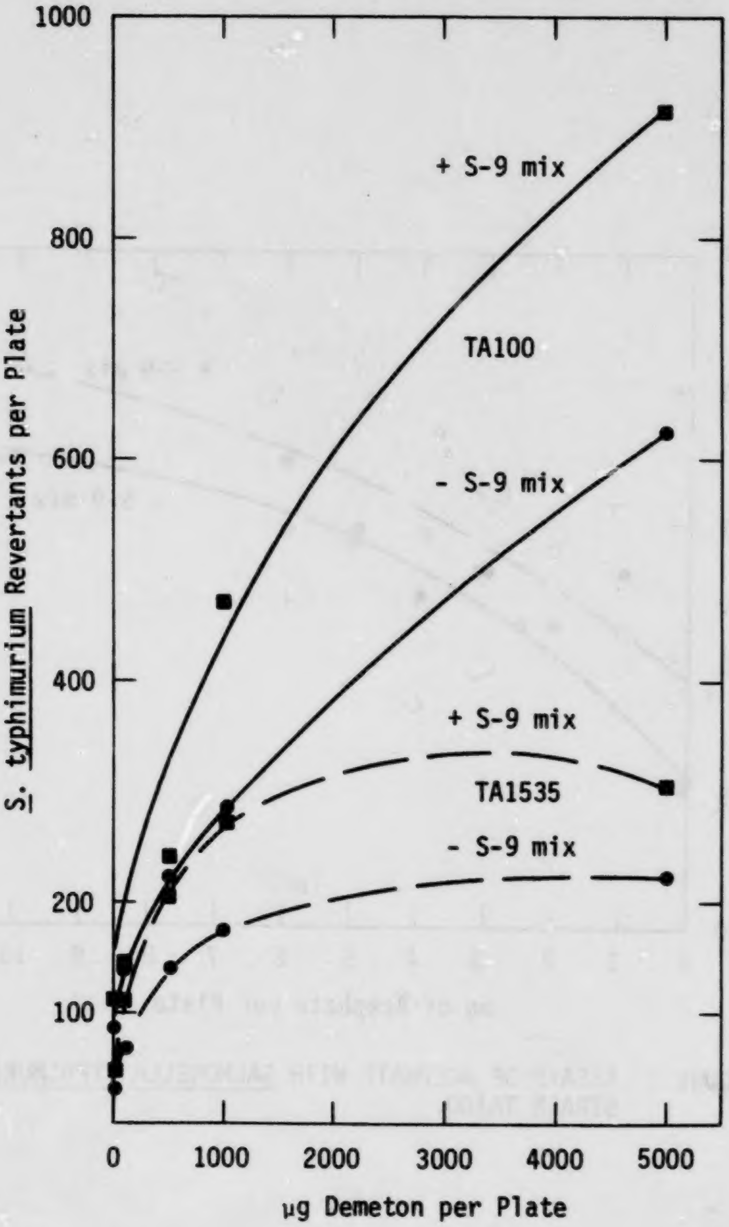


FIGURE 2 ASSAYS OF DEMETON WITH SALMONELLA TYPHIMURIUM STRAIN TA1535 and TA100.

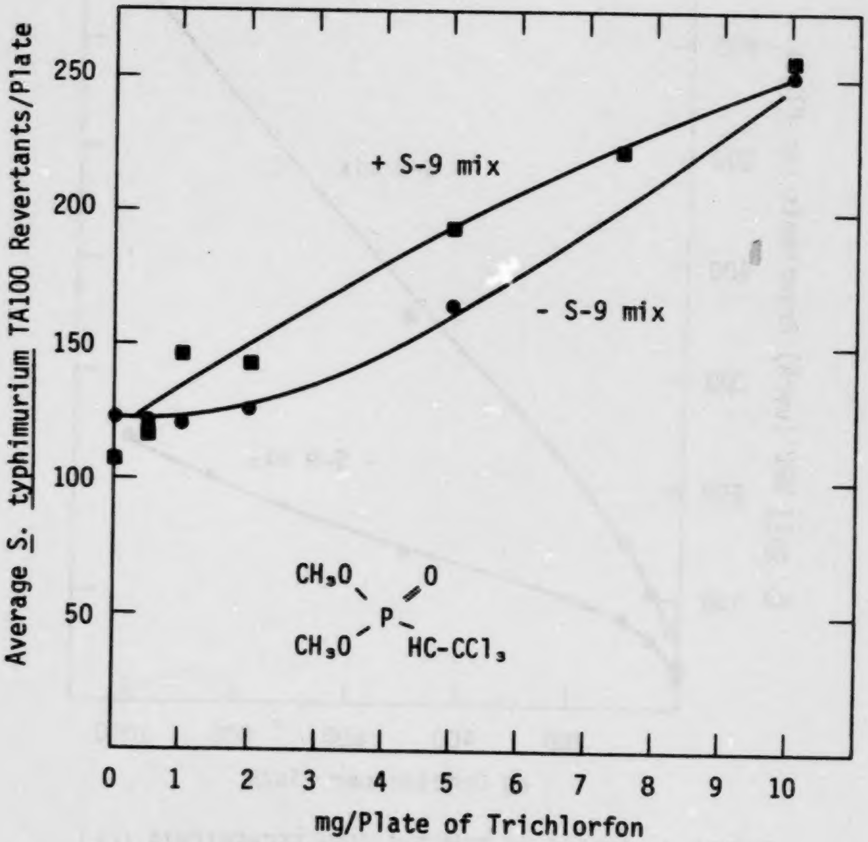


FIGURE 3 ASSAYS OF TRICHLORFON WITH SALMONELLA TYPHIMURIUM STRAIN TA100.

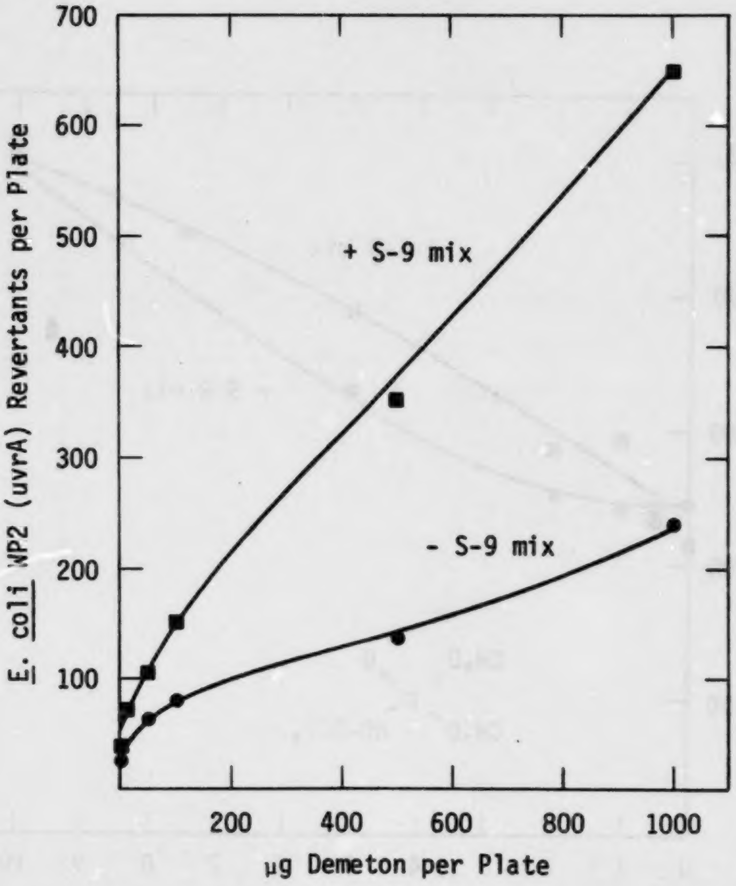


FIGURE 4 ASSAYS OF DEMETON WITH *ESCHERICHIA COLI* STRAIN WP2.

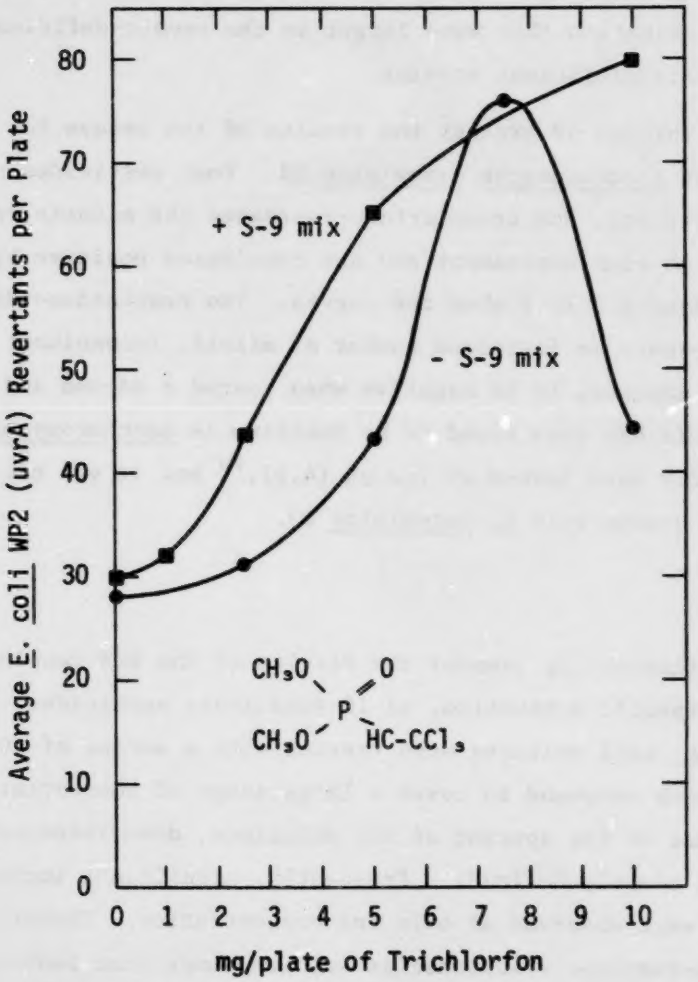


FIGURE 5 ASSAYS OF TRICHLORFON WITH ESCHERICHIA COLI STRAIN WP2.

Toxic chemicals that do not act by damaging DNA (e.g., chloramphenicol) should give equal zones of toxicity on both repair-proficient and repair-deficient strains. However, a given concentration of a chemical that is genotoxic may be toxic for a repair-deficient strain but not for a strain that effectively repairs its DNA. Dicamba, 2,4-D acid, 2,4-DB acid, demeton, and propanil gave positive responses in these assays; i.e., each gave zones of inhibition that were larger in the repair-deficient strains than in the repair-proficient strains.

Tables 50 through 68 present the results of the assays for mitotic recombination in Saccharomyces cerevisiae D3. Four pesticides--demeton, crotoxyphos, acephate, and trichlorfon--increased the mitotic recombination frequency in each experiment and are considered positive by these procedures. Figures 6 to 9 show the curves. Two pesticides--diazinon and disulfoton--gave an increased number of mitotic recombinants in one experiment but appeared to be negative when tested a second and third time. 2,4-D acid has been found to be positive in Saccharomyces cerevisiae D4, D5, and RAD18 when tested at low pH (4.3),[28] but it was not recombinogenic in our assays with S. cerevisiae D3.

UDS Assays

Table 69 through 119 present the results of the UDS testing, with and without metabolic activation, of 18 substitute pesticides. For the initial testing, cell cultures were treated with a series of 10-fold dilutions of each compound to cover a large range of concentrations. However, because of the spacing of the dilutions, dose-response relationships were not clearly defined. Frequently, significant increases in ^{3}H-TdR uptake were observed at only one concentration. Therefore, to establish dose-response relationships for compounds that indicated a significant (statistically significant at the 95% confidence level) increase in UDS in the initial tests, additional tests were performed using narrower concentration ranges and higher concentrations of test chemical, if necessary.

The first test of demeton without metabolic activation (Table 68) indicated a statistically significant elevation in ^{3}H-TdR incorporation

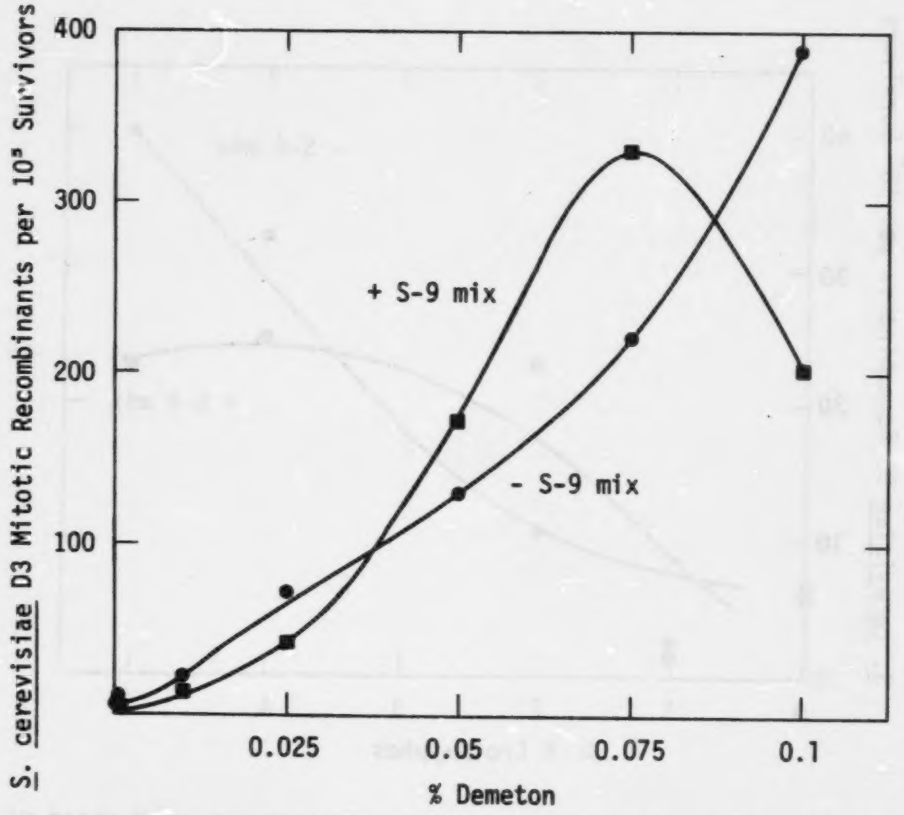


FIGURE 6 ASSAYS OF DEMETON WITH SACCHAROMYCES CEREVISIAE D3.

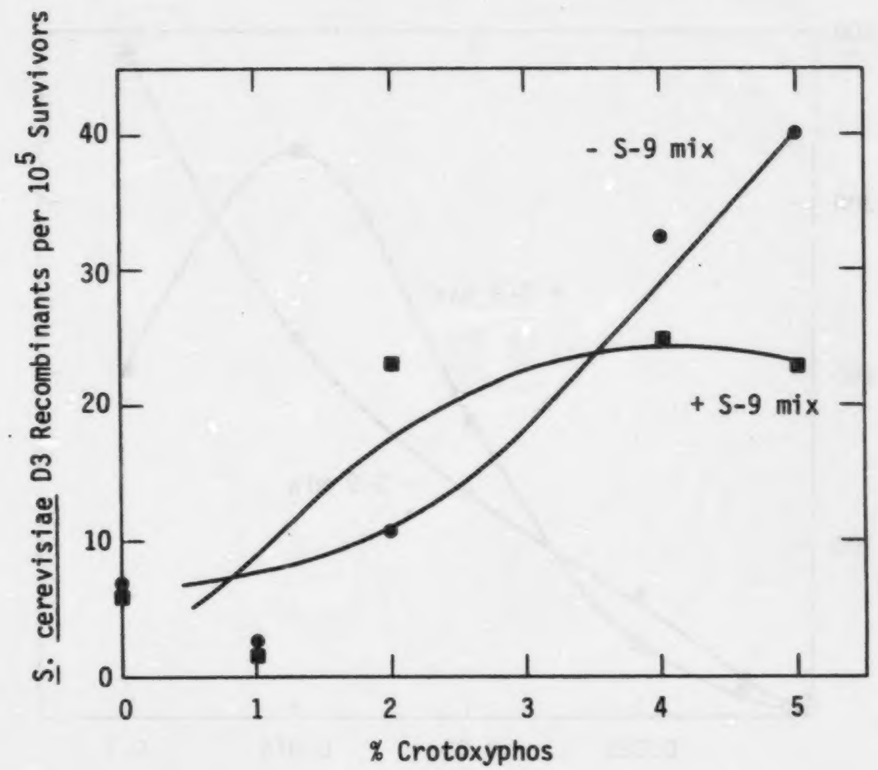


FIGURE 7 ASSAYS OF CROTOXYPHOS WITH SACCHAROMYCES CEREVISIAE D3.

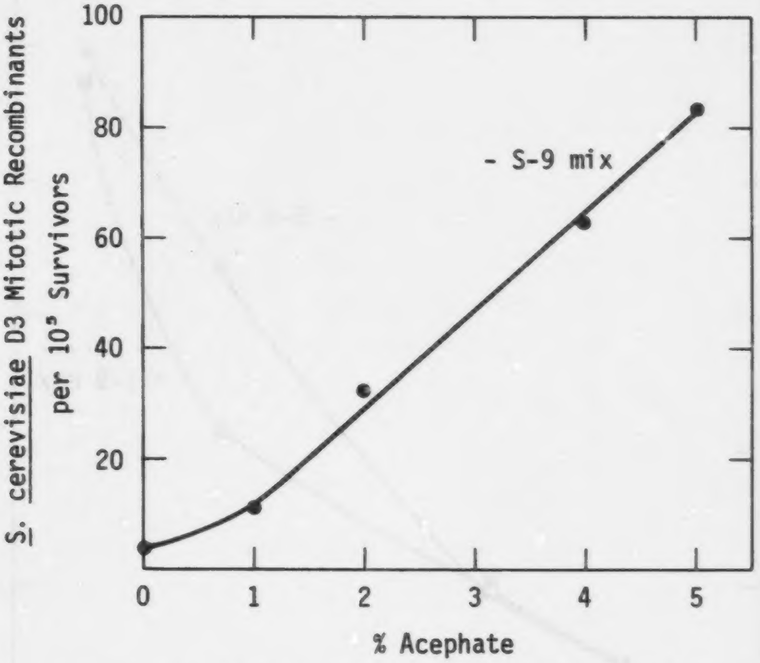


FIGURE 8 ASSAY OF ACEPHATE WITH *SACCHAROMYCES CEREVISIAE* D3 WITHOUT METABOLIC ACTIVATION.

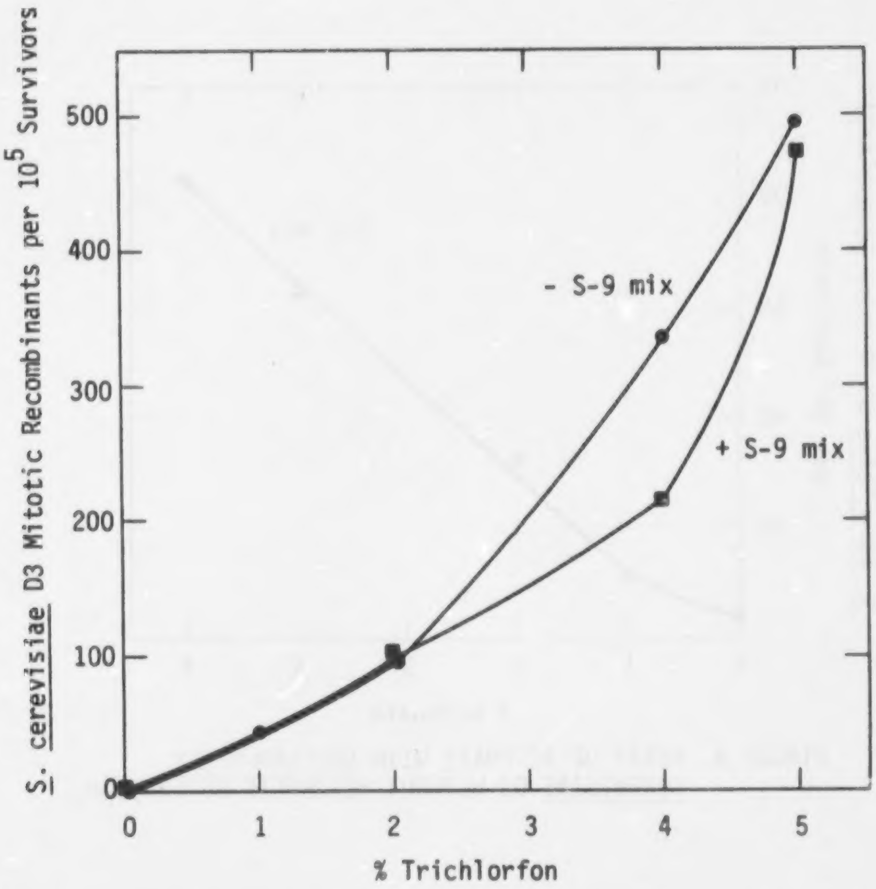


FIGURE 9 ASSAYS OF TRICHLORFON WITH SACCHAROMYCES CEREVISIAE D3.

at the 99% confidence level ($H = 25.81 \cong \chi_5^2 > 15.09$); however, a dose-response relationship was not observed in this test. A second test of demeton under similar conditions, but with a narrower dose range (Table 69), again indicated a statistically significant (99% confidence) response ($F_{5,30} = 31.17 > 3.70$), with a characteristic dose-response relationship. Similarly, the initial test of demeton with metabolic activation (Table 70) indicated an increase in UDS ($F_{5,30} = 53.58 > 3.70$) without an apparent dose-response relationship. A repeat of this test (Table 71) also indicated a statistically significant (99% condidence) response ($F_{5,29} = 14.11 > 3.73$). In addition, this assay provided evidence of the existence of a dose-related response to demeton in the presence of the metabolic activation preparation. Figure 10 depicts the results of the testing of demeton with and without metabolic activation. The dose-response curves are illustrative of the characteristic differences between the testing of direct-acting compounds in the assay with metabolic activation and the testing in the assay without it. The two most striking features are: (1) an order of magnitude difference exists between the concentration ranges at which the dose-response curves are observed, with the concentration range for testing with metabolic activation being higher; and (2) the extent of UDS is diminished in the assay with metabolic activation. Three nonexclusive hypotheses can account for these variations. The first is that demeton is--at least in part--enzymatically inactivated by the metabolic activation preparation. Alternatively, a nonspecific interaction occurs between demeton and the activation preparation that reduces the effective concentration to which the cells are exposed. The third hypothesis is that the reduced duration of exposure to the compound (the exposure duration with metabolic activation is one-third that used without metabolic activation) accounts for the decreased activity. Unfortunately, the data presented do not offer any evidence that would support one of these hypotheses over the others.

The initial results of testing acephate without metabolic activation (Table 73) suggested that if this compound increases UDS, the response occurs at concentrations above 100 µg/ml and the maximum response could be obtained at some concentration above 1000 µg/ml. An additional test with this compound (Table 73) confirmed this. Figure 11 depicts the results of the first and second tests of acephate. We obtained a

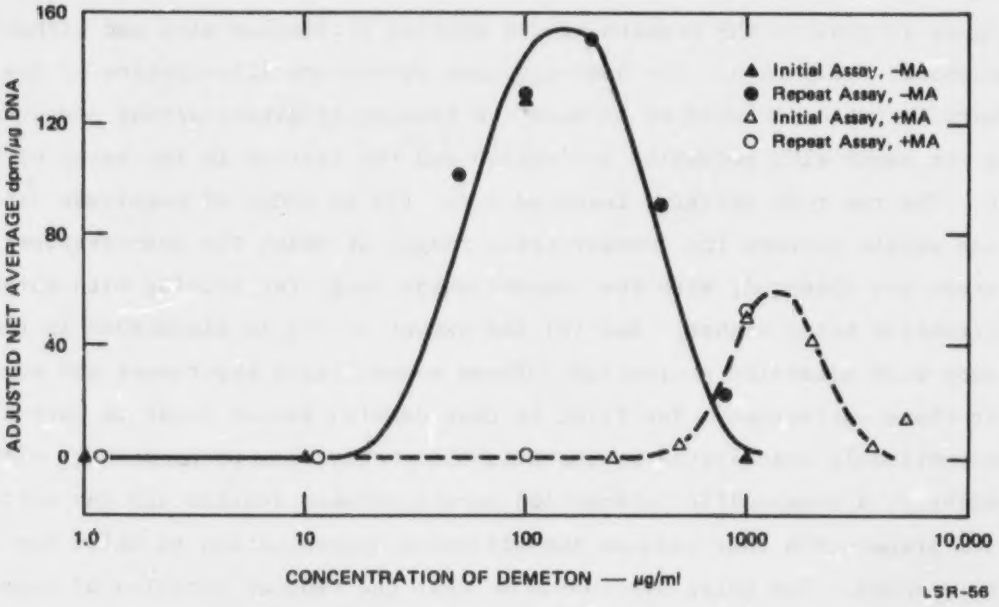
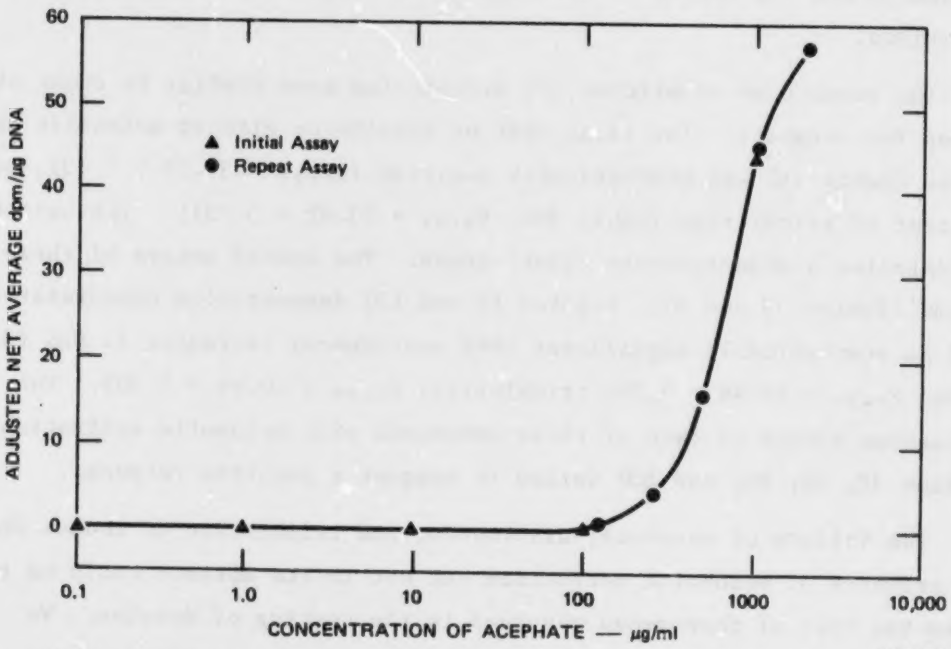


FIGURE 10 UNSCHEDULED DNA SYNTHESIS ASSAYS OF DEMETON



LSR-57

FIGURE 11 UNSCHEDULED DNA SYNTHESIS ASSAYS OF ACEPHATE WITHOUT METABOLIC ACTIVATION

statistically positive (99% confidence) response at 2000 μg/ml ($F_{5,30}$ = 7.99 > 3.70). Because of the normal variability between the cultures used for each assay, the increases in incorporation of ^{3}H-TdR observed between 250 μg/ml and 1000 μg/ml were not statistically significant at the 99% confidence level. However, the second test clearly demonstrated a dose-response relationship as well as the reproducibility of the initial test results. The first and second tests of acephate with metabolic activation (Tables 74 and 75) failed to indicate an increase in UDS under these test conditions.

The results on disulfoton and trichlorfon were similar to those observed for acephate. The first test of disulfoton without metabolic activation (Table 76) was statistically positive ($F_{5,29}$ = 39.79 > 3.73), as was the test of trichlorfon (Table 80; $F_{5,29}$ = 13.42 > 3.73); yet neither demonstrated a dose-response relationship. The second assays of these compounds (Tables 77 and 81; Figures 12 and 13) demonstrated dose-related as well as statistically significant (99% confidence) increases in UDS (disulfoton, $F_{5,30}$ = 18.40 > 3.70; trichlorfon, $F_{5,30}$ = 10.44 > 3.70). The first and second assays of each of these compounds with metabolic activation (Tables 78, 79, 82, and 83) failed to suggest a positive response.

The failure of acephate, disulfoton, and trichlorfon to induce UDS in the presence of metobolic activation but not in its absence could be related to the type of phenomenon observed in the testing of demeton. We believe that if a response could be observed in assays with acephate, disulfoton, or trichlorfon with metabolic activation, it would occur in a concentration range roughly an order of magnitude greater than that at which it occurs without metabolic activation. Since the maximum concentrations tested, both with and without metabolic activation, for each of these compounds were near the maximum feasible under the constraints of the solubilities of the compound and the procedures described in "Dilution of Compounds", probably none of these three compound could be tested at high enough concentrations with metabolic activation to obtain a positive response.

The testing of dicamba and fensulfothion (Tables 84 to 92) indicated that although neither was positive without metabolic

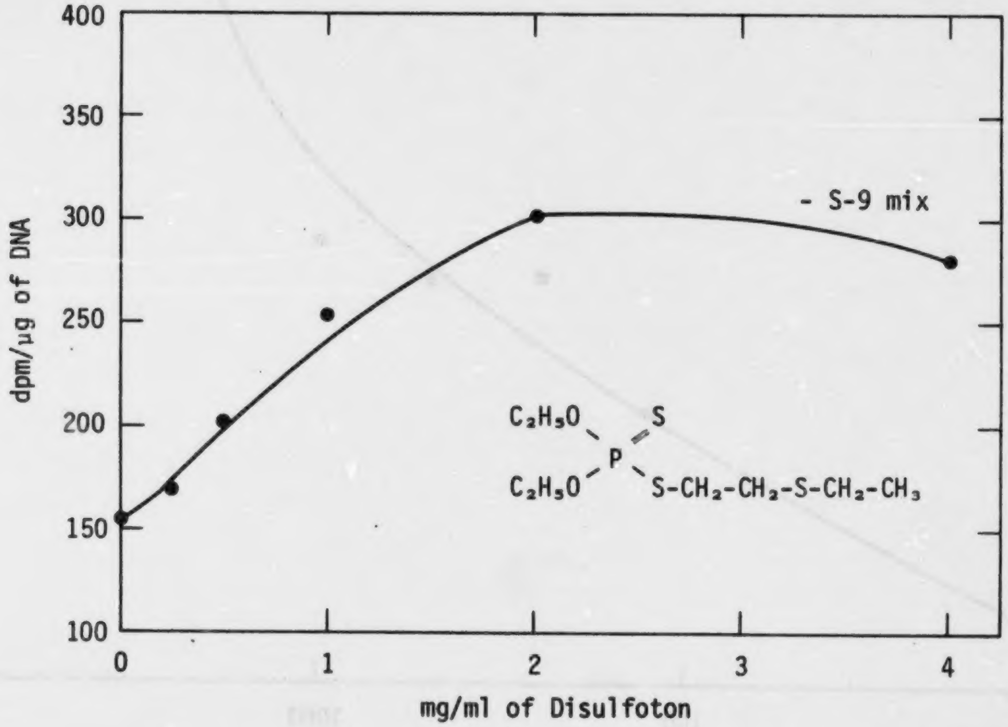


FIGURE 12 UNSCHEDULED DNA SYNTHESIS ASSAYS OF DISULFOTON WITHOUT METABOLIC ACTIVATION.

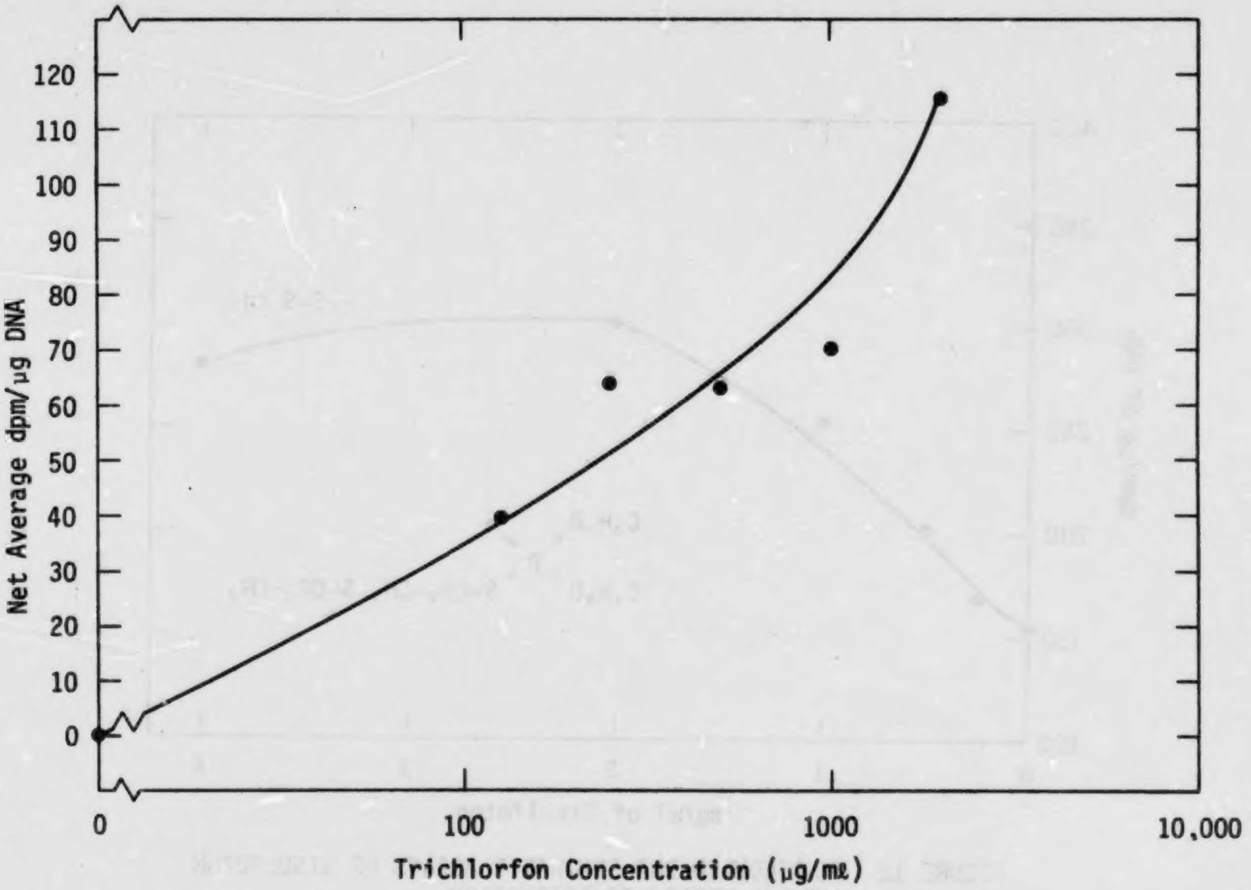


FIGURE 13 UNSCHEDULED DNA SYNTHESIS ASSAYS OF TRICHLORFON WITHOUT METABOLIC ACTIVATION

activation, both initially appeared to induce UDS in the presence of metabolic activation. In the initial tests with metabolic activation, no UDS effects were observed for dicamba below 100 μg/ml, although a statistically significant response was observed at 1000 μg/ml (Table 84). No effects were observed for fensulfothion below 1.0 μg/ml, and a broad plateau of positive responses was obtained at concentrations between 1 and 1000 μg/ml (Table 89). However, the retesting of these compounds with metabolic activation (Tables 86-88, 91 and 92), failed to indicate either a dose-response relationship or a positive response for either compound. Thus, the initial results were not confirmed, and we conclude that these two compounds are unable to induce UDS.

Tests of endrin without metabolic activation gave negative results (Table 93). The results of initial testing with activation (Table 94) suggested an increase at the highest concentration tested (1000 μg/ml), but it was not statistically positive. Therefore, additional testing of endrin was performed in an attempt to show a dose-response relationship (Tables 95 and 96). We attempted to achieve a maximum concentration at 3000 μg/ml; however, due to the incomplete solubility of this compound in DMSO at such a high concentration, it is doubtful that a true solution was obtained. Although the first retest (Table 97) again indicated an apparent, but statistically insignificant, elevation of ^{3}H-TdR incorporation at the highest concentration tested, it did not resolve the question of whether a dose-response relationship can be observed for the effects of endrin with metabolic activation. Therefore, a third assay was conducted using modified dilution procedures that would allow us to achieve the higher test concentrations of endrin. The results of this test (Table 96) failed to indicate that this compound is capable of inducing UDS even when higher concentrations are achieved. Hence, we conclude that the results of the UDS testing of endrin are negative.

The tests of aspon (Tables 97 and 98), carbofuran (Tables 99 and 100), crotoxyphos (Tables 101 and 102), 2,4-D acid (Tables 103 and 104), 2,4-DB acid (Tables 95 and 96), diazinon (Tables 107 and 108), fonofos (Tables 109 and 110), ethion (Tables 111 and 112), methoxychlor (Tables 113 and 114), siduron (Tables 115 and 116), and propanil (Tables 117 and 118), both with and without metabolic activation, all failed to indicate increased ^{3}H-TdR incorporation. Consequently, we conclude that none of these compounds increases UDS either in the presence or in the absence of metabolic activation.

CONCLUSIONS

Nine of the 18 pesticides tested in Phase II were mutagenic or genotoxic in *in vitro* assays. Two of the pesticides had a broad spectrum of activity: demeton and trichlorfon increased reverse mutation in *Salmonella* and *E. coli* WP2. Acephate caused a small but reproducible increase in *S. typhimurium* TA100 revertants, increased mitotic recombination in *S. cerevisiae* D3, and increased unscheduled DNA synthesis. Demeton also was more toxic to DNA repair-deficient strains of *E. coli* and *B. subtilis* than to repair-proficient strains. Based on these results, substituting these pesticides for other pesticides currently in use would be unwise unless long-term animal tests indicate that they do not pose a carcinogenic or mutagenic risk.

Crotoxyphos, 2,4-D acid, 2,4-DB acid, dicamba, disulfoton, and propanil exhibited a narrow range of effects. The observation of increased toxicity to *B. subtilis* strain M45 compared with toxicity to strain H17 by 2,4-D acid is in contrast to the absence of such an effect reported by Shirasu.[29] Interestingly, none of these six pesticides was mutagenic in *Salmonella*. Perhaps testing them in other *in vitro* assays (cytogentics, sister chromatid exchange, transformation or mutation of mammalian cells) would be appropriate before drawing any conclusions about their potential effect on the environment.

Nine pesticides--aspon, carbofuran, diazinon, endrin, ethion, fensulfothion, fonofos, methoxychlor, and siduron--were not active in any of the assays. These results indicate that these pesticides are good candidates to substitute for pesticides that have been found to be harmful to the environment.

Table 1

EIGHTEEN PESTICIDES EVALUATED BY SRI INTERNATIONAL FOR MUTAGENICITY

Common Name	Trade Name	Manufacturer	Purity	Batch or Lot No.	Supplier
Acephate	Orthene	Chevron Chem.	Tech. 93.5%	5X-7562	Battelle
Aspon	Tetra-N-propyl dithionopyrophosphate	Stauffer Chem. Co.	Tech. 93.0%	3714-35	Battelle
Carbofuran	Furadan	Niagara Chem.	Tech.	C-4717-54A	Battelle
Crotoxyphos	Ciodrin	Shell Chem. Co.	Tech. 85%	Batch 11-PPC-32	Battelle
2,4-D acid		Rhodia Inc.	98.6%	610123	EPA
2,4-DB acid	Embutox	Rhodia Inc.	98.9%	610123	EPA
Demeton	Systox	Chemagro	Tech. 85%		Battelle
Diazinon	MG8; Bosudin; Neocidol	CIBA-Geigy	Tech.	Batch FL-741305	Battelle
Dicamba	Banvel; Mediben	Velsicol	Tech.		Battelle
Disulfoton	Di-Syston	Chemagro	Tech.	Batch 5-08-5110	Battelle
Endrin	Exp Insecticide 209	J. Hyman & Co.	99.5%		EPA
Ethion	Nialate	FMC	Tech.	Lot No. 3381-5	EPA
Fensulfothion	Dasanit; Terracur-P	Chemagro	Tech.	Batch 5-10-0004	Battelle
Fonofos	Dyfonate	Stauffer Chem. Co.	96.9%	CBA0804	Battelle
Methoxychlor	Marlate	E. I. du Pont de Nemours & Co.	Tech.	6543-108	Battelle
Propanil	Stam Tech	Rohm and Haas	88.0%	6-2402	EPA
Siduron	Tupersan	du Pont	Tech. 96.7%	Lot I-70715-C	Battelle
Trichlorfon	Dylox; Dipterex	Chemagro	Tech.	Batch 5-00-7003	Battelle

Table 2

IN VITRO MUTAGENISIS: SUMMARY DATA FOR EPA PESTICIDES

Pesticide	*Salmonella typhimurium* (His$^+$ Reversion)		*Escherichia coli* WP2 (Try$^+$ Reversion)		*Saccharomyces cerevisiae* D3 (Mitotic Recombinants)		*Escherichia coli* Relative Toxicity	*Bacillus subtilis* Relative Toxicity	UDS (DNA Repair)	
	-MA	+MA	-MA	+MA	-MA	+MA			-MA	+MA
Acephate	+*	+	-	-	+	+	-	-	+	-
Aspon	-	-	-	-	-	-	-	-	-	-
Carbofuran	-	-	-	-	-	-	-	-	-	-
Crotoxyphos	-	-	-	-	+	+	-	-	-	-
2,4-D acid	-	-	-	-	-	-	+	+	-	-
2,4-DB acid	-	-	-	-	-	-	+	+	-	-
Demeton	+	+	+	+	+	+	+	+	+	+
Diazinon	-	-	-	-	-	-	-	-	-	-
Dicamba	-	-	-	-	-	-	+	+	-	-
Disulfoton	-	-	-	-	-	-	-	-	+	-
Endrin	-	-	-	-	-	-	-	-	..	-
Ethion	-	-	-	-	-	-	-	-	-	-
Fensulfothion	-	-	-	-	-	-	-	-	-	-
Fonofos	-	-	-	-	-	-	-	-	-	-
Methoxychlor	-	-	-	-	-	-	-	-	-	-
Propanil	-	-	-	-	-	-	+	+	-	-
Siduron	-	-	-	-	-	-	-	-	-	-
Trichlorfon	+	+	+	+	+	+	-	-	+	-

---

*Positive response, +; negative response, -.

Table 3

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF ACEPHATE

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		28	10	10	56	124
	+		36	11	9	54	97
Positive control 2-Anthramine	-	20	36				
	+	20	480	312	2000	3500	1680
Acephate	-	1	36	7	7	61	185
	-	10	40	11	6	65	151
	-	50	38	11	5	62	165
	-	100	38	12	8	58	154
	-	500	38	10	8	56	169
	-	1000	37	14	10	56	154
	+	1	34	12	14	49	60
	+	10	26	9	9	53	136
	+	50	32	15	9	48	132
	+	100	22	18	11	49	130
	+	500	34	13	11	63	87
	+	1000	20	17	8	55	121

Table 4

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF ACEPHATE

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		14	18	14	22	121
	+		14	7	18	19	127
Positive controls							
β-Propiolactone	-	50	633	365			617
2-Anthramine	-	50			18	38	
	+	50			2620	2956	
Acephate	-	10	18	12	15	11	109
	-	50	21	16	18	14	95
	-	100	15	10	13	18	103
	-	500	13	17	18	29	127
	-	1000	28	12	21	24	108
	-	5000	16	18	15	14	166
	+	10	11	13	18	29	150
	+	50	12	16	18	12	100
	+	100	16	13	10	30	112
	+	500	17	16	27	33	138
	+	1000	5	12	16	42	117
	+	5000	15	11	17	25	177

Table 5

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF ACEPHATE
EXPERIMENTS 3 AND 4

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate TA100
	EXPERIMENT 3		
Negative control	-		126
	+		150
Positive control 2-Anthramine	+	2.5	558
Acephate	-	1000	166
	-	2000	156
	-	3000	165
	-	4000	161
	-	5000	172
	-	10000	187
	+	1000	155
	+	2000	169
	+	3000	166
	+	4000	172
	+	5000	169
	+	10000	211
	EXPERIMENT 4		
Negative control	-		138
	+		129
Positive control 2-Anthramine	+	2.5	1368
Acephate	-	2500	156
	-	5000	173
	-	6000	186
	-	8000	165
	-	10000	166
	+	2500	122
	+	5000	155
	+	6000	174
	+	8000	170
	+	10000	190

Table 6

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF ASPON

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		16	5	10	2	140
	+		10	6	15	7	152
Positive controls							
β-Propiolactone	-	50	1160				1682
2-Anthramine	-	20			11	7	
	+	20			2204	180	
9-Aminoacridine	-	100		1218			
Aspon	-	10	14	9	8	7	128
	-	50	16	4	5	4	119
	-	100	12	9	9	5	126
	-	500	11	6	10	8	134
	-	1000	11	6	10	7	130
	-	5000	8	8	6	6	127
	+	10	9	7	18	3	147
	+	50	10	7	16	5	127
	+	100	8	6	18	5	136
	+	500	7	13	19	9	109
	+	1000	8	7	14	12	116
	+	5000	11	10	12	6	114

Table 7

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF ASPON

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		16	8	18	26	143
	+		19	12	23	34	154
Positive control 2-Anthramine	-	50			14	42	
	+	50	450	320	3000	3800	3000
Aspon	-	10	16	11	15	30	101
	-	50	19	13	16	27	132
	-	100	14	10	8	27	125
	-	500	15	11	10	33	127
	-	1000	17	14	16	34	102
	-	5000	22	11	13	30	107
	+	10	18	11	24	39	132
	+	50	22	16	24	39	99
	+	100	19	11	20	34	117
	+	500	21	9	17	41	102
	+	1000	15	15	13	34	84
	+	5000	22	7	17	39	106

Table 8

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF CARBOFURAN

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		17	12	15	25	144
	+		12	10	22	30	126
Positive controls							
2-Anthramine	-	20			21		
	+	20		180	1860	491	
N-Methyl-N'-nitro-N-nitrosoguanidine	-	2	366				562
Carbofuran	-	1	4	10	12	24	141
	-	10	4	9	11	27	123
	-	50	5	7	11	27	122
	-	100	C*	9	12	41	122
	-	500	C	7	14	36	127
	-	1000	C	12	14	27	134
	+	1	7	10	24	28	142
	+	10	18	10	24	25	126
	+	50	15	9	15	26	126
	+	100	10	14	17	27	133
	+	500	C	10	18	24	132
	+	1000	C	13	14	23	141

---

* C, contaminated.

Table 9

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF CARBOFURAN

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		30	9	29	44	114
	+		20	17	32	39	152
Positive control 2-Anthramine	-	20				86	
	+	20	720	223	1080	1220	1200
Carbofuran	-	10	22	9	13	20	134
	-	50	39	15	17	31	123
	-	100	35	10	15	39	138
	-	500	38	13	12	25	147
	-	1000	39	12	18	29	127
	-	5000	42	14	17	21	122
	+	10	13	12	29	46	128
	+	50	15	18	28	49	114
	+	100	11	19	39	35	129
	+	500	18	16	33	56	143
	+	1000	13	20	29	42	173
	+	5000	16	18	30	36	119

Table 10

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*

OF CROTOXYPHOS

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		8	10	10	25	137
	+		12	13	13	30	116
Positive control							
N-Methyl-N'-nitro-N-nitrosoguanidine	-	2	196				488
2-Anthramine	-	20			38		
	+	20		457	3500	3000	
Crotoxyphos	-	1	12	8	16	29	116
	-	10	13	12	13	19	99
	-	50	11	10	13	38	100
	-	100	11	11	11	27	89
	-	500	13	12	6	17	99
	-	1000	11	11	12	17	109
	+	1	9	13	14	20	95
	+	10	14	8	8	31	131
	+	50	13	12	12	30	114
	+	100	12	7	13	32	99
	+	500	12	10	15	25	139
	+	1000	8	6	15	26	101

Table 11

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF CROTOXYPHOS

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		19	13	20	25	84
	+		13	17	19	30	107
Positive control 2-Anthramine	-	20			18		
	+	20	223	516	759	491	1250
Crotoxyphos	-	10	16	18	18	31	84
	-	50	16	10	20	17	102
	-	100	22	7	12	18	86
	-	500	20	13	16	24	88
	-	1000	19	3	12	19	91
	-	5000	18	4	16	18	104
	+	10	10	11	22	24	87
	+	50	15	8	29	17	75
	+	100	18	18	25	25	94
	+	500	12	9	19	25	91
	+	1000	10	8	20	22	87
	+	5000	3	6	16	25	112

Table 12

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*
OF 2,4-D ACID

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		19	7	12	22	157
	+		20	8	20	31	104
Positive controls							
β-Propiolactone	-	50	280				375
2-Anthramine	-	10			14	32	
	+	10			1545	1661	
9-Aminoacridine	-	100		375			
2,4-D Acid	-	10	23	7	10	23	115
	-	50	13	11	15	15	88
	-	100	23	9	5	27	115
	-	500	16	4	5	22	90
	-	1000	9	7	9	23	94
	-	5000	3	2	0	0	46
	+	10	10	6	8	32	100
	+	50	6	8	8	24	105
	+	100	10	12	10	29	69
	+	500	7	11	16	26	107
	+	1000	13	5	12	27	92
	+	5000	6	5	4	10	47

Table 13

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF 2,4-D ACID

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		5	11	15	26	171
	+		6	15	24	40	193
Positive control							
β-Propiolactone	-	50	85				288
2-Anthramine	-	10			6	26	
	+	10		167	1224	847	
2,4-D Acid	-	10	9	10	6	16	113
	-	50	9	11	7	15	111
	-	100	5	8	4	19	95
	-	500	7	5	9	12	85
	-	1000	4	7	4	12	75
	-	5000	T*	T	T	T	T
	+	10	12	12	11	21	109
	+	50	7	8	12	24	123
	+	100	7	7	13	29	134
	+	500	8	8	18	12	108
	+	1000	9	7	13	23	104
	+	5000	T	5	9	T	T

---

* T, toxic.

Table 14

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* OF 2,4-DB ACID

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		19	5	12	22	157
	+		20	8	20	31	104
Positive controls							
β-Propiolactone	-	50	280				375
2-Anthramine	-	10			14	32	
	+	10			1545	1661	
2-Aminoacridine	-	100		374			
2,4-DB Acid	-	10	20	6	8	15	104
	-	50	25	12	12	22	85
	-	100	16	5	7	16	100
	-	500	18	2	T*	15	95
	-	1000	11	0	T	T	77
	-	5000	T	1	T	T	67
	+	10	12	8	9	24	98
	+	50	11	5	7	21	94
	+	100	17	4	4	31	94
	+	500	15	4	11	28	77
	+	1000	14	4	8	10	T
	+	5000	16	2	8	10	T

---

* T, toxic.

Table 15

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF 2,4-DB ACID
EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		5	11	15	26	171
	+		6	15	24	40	193
Positive control							
β-Propiolactone	-	50	85				288
2-Anthramine	-	10			6	26	
	+	10		167	1224	847	
2,4-DB Acid	-	10	11	4	6	11	117
	-	50	10	12	6	19	133
	-	100	12	9	5	19	141
	-	500	12	6	8	24	99
	-	1000	T*	1	T	T	90
	-	5000	T	T	T	T	T
	+	10	13	7	15	30	132
	+	50	6	8	10	32	139
	+	100	10	12	18	24	136
	+	500	6	5	9	32	121
	+	1000	9	7	12	27	102
	+	5000	3	T	T	T	T

---

* T , toxic.

Table 16

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF DEMETON
EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		8	10	10	90	137
	+		12	13	13	97	116
Positive control							
N-Methyl-N'-nitro-N-nitrosoguanidine	-	2	196				488
2-Anthramine	-	20			38	135	
	+	20		457	3500	3000	
Demeton	-	1	10	3	9	94	99
	-	10	22	6	13	76	127
	-	50	36	15	9	73	147
	-	100	83	9	9	81	167
	-	500	224	15	7	87	227
	-	1000	311	6	10	79	295
	+	1	13	8	13	99	104
	+	10	30	5	12	78	159
	+	50	135	8	12	86	208
	+	100	140	16	9	92	190
	+	500	518	10	14	79	372
	+	1000	656	9	13	79	551

Table 17

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF DEMETON

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		27	13	20	67	84
	+		44	17	19	89	107
Positive control 2-Anthramine	-	20			18	137	
	+	20	155	516	759	1342	1250
Demeton	-	10	60	15	15	92	114
	-	50	59	10	18	83	120
	-	100	64	11	11	85	137
	-	500	139	11	21	94	219
	-	1000	172	11	20	87	282
	-	5000	221	8	8	73	625
	+	10	44	12	25	107	79
	+	50	93	21	32	94	166
	+	100	109	12	21	101	145
	+	500	201	14	22	67	240
	+	1000	275	12	31	82	469
	+	5000	303	15	23	47	914

Table 18

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF DIAZINON
EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		14	8	7	22	163
	+		19	15	27	44	176
Positive control							
2-Anthramine	-	20			39	27	
	+	20		200	2880	3840	
β-Propiolactone	-	50	1980				2520
Diazinon	-	1	24	7	16	32	141
	-	10	24	5	18	17	136
	-	50	19	11	8	24	147
	-	100	20	14	10	21	138
	-	500	21	13	10	18	123
	-	1000	21	8	12	26	139
	+	1	5	5	19	22	187
	+	10	10	12	21	21	148
	+	50	14	12	20	23	161
	+	100	9	15	17	25	119
	+	500	7	9	8	26	143
	+	1000	15	10	14	20	127

Table 19

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF DIAZINON

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		30	13	25	25	131
	+		17	14	17	30	124
Positive control							
N-Methyl-N'-nitro-N-nitrosoguanidine	-	2	186				494
2-Anthramine	-	20			27		
	+	20		485	2400	491	
Diazinon	-	10	14	10	8	30	124
	-	50	13	20	11	26	157
	-	100	6	12	4	36	154
	-	500	11	13	14	29	131
	-	1000	15	13	4	33	116
	-	5000	14	10	9	21	147
	+	10	14	11	16	16	112
	+	50	10	14	15	27	105
	+	100	16	19	12	20	112
	+	500	10	12	11	27	82
	+	1000	16	13	9	25	134
	+	5000	14	8	10	23	128

Table 20

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF DICAMBA

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		28	10	10	56	124
	+		36	11	9	54	97
Positive control 2-Anthramine	-	20	36				
	+	20	480	312	2000	3500	1680
Dicamba	-	1	38	8	8	44	168
	-	10	43	12	8	65	152
	-	50	44	18	8	53	114
	-	100	37	18	11	45	126
	-	500	38	10	7	55	153
	-	1000	40	7	9	37	135
	+	1	28	9	7	55	77
	+	10	41	15	5	44	150
	+	50	32	10	4	47	131
	+	100	34	8	8	44	157
	+	500	37	6	6	38	116
	+	1000	26	9	6	41	149

Table 21

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF DICAMBA

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		13	14	19	24	94
	+		11	13	19	32	95
Positive controls							
9-Aminoacridine	-	100		128			
2-Anthramine	-	50			20	40	
	+	50	225		2453	2960	1950
Dicamba	-	10	7	15	10	30	92
	-	50	22	12	16	34	87
	-	100	18	14	12	36	95
	-	500	16	14	17	32	68
	-	1000	12	12	12	26	75
	-	5000	0	0	0	0	0
	+	10	19	13	26	24	87
	+	50	12	12	25	47	93
	+	100	9	15	24	38	99
	+	500	21	11	23	33	84
	+	1000	15	13	17	29	96
	+	5000	8	3	23	31	75

Table 22

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* OF DICAMBA

EXPERIMENT 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate TA100
Negative control	-		109
	+		113
Positive control 2-Anthramine	+	2.5	212
Dicamba	-	1	98
	-	10	110
	-	50	99
	-	100	105
	-	500	139
	-	1000	110
	-	2000	80
	-	3000	T*
	-	4000	T
	-	5000	T
	+	1	104
	+	10	103
	+	50	110
	+	100	121
	+	500	101
	+	1000	89
	+	2000	100
	+	3000	87
	+	4000	95
	+	5000	82

---

* T, toxic.

Table 23

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*

OF DISULFOTON

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		14	8	7	22	163
	+		10	15	27	44	176
Positive controls							
2-Anthramine	-	20			39	27	
	+	20		200	2880	3840	
β-Propiolactone	-	50	1980				2520
Disulfoton	-	1	12	13	10	22	116
	-	10	16	7	15	19	136
	-	50	19	9	7	18	112
	-	100	17	12	11	15	109
	-	500	18	6	10	23	146
	-	1000	23	6	12	26	133
	+	1	9	13	17	32	121
	+	10	10	15	20	34	137
	+	50	7	9	15	41	121
	+	100	10	10	16	22	128
	+	500	22	9	14	34	135
	+	1000	23	13	17	29	152

Table 24

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF DISULFOTON

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		29	15	30	44	226
	+		25	18	33	51	251
Positive control							
AF2	-	0.5				190	720
2-Anthramine	-	20	1330	700	3800	4940	8000
Disulfoton	-	10	25	17	31	24	178
	-	50	29	15	30	49	196
	-	100	30	16	33	32	192
	-	500	25	18	29	51	228
	-	1000	44	22	39	50	206
	-	5000	57	18	14	50	224
	+	10	35	17	32	55	142
	+	50	21	17	37	53	146
	+	100	21	21	42	55	214
	+	500	24	16	33	53	258
	+	1000	38	17	36	45	172
	+	5000	48	22	34	32	202

Table 25

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF DISULFOTON

EXPERIMENT 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate TA100
Negative control	-		126
	+		150
Positive control 2-Anthramine	+	2.5	558
Disulfoton	-	10	143
	-	50	125
	-	100	148
	-	500	130
	-	1000	138
	-	2000	143
	-	3000	145
	-	4000	150
	-	5000	164
	+	10	143
	+	50	124
	+	100	132
	+	500	129
	+	1000	149
	+	2000	148
	+	3000	155
	+	4000	148
	+	5000	141

Table 26

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF ENDRIN

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		167	10	30	40	127
	+		93	21	30	59	105
Positive controls							
β-Propiolactone	-	50	277				386
2-Anthramine	-	10			28	41	
	+	10			1843	2281	
Endrin	-	1	100	19	31	28	145
	-	10	85	12	19	39	142
	-	50	104	16	8	29	160
	-	100	89	14	22	39	146
	-	500	107	15	22	34	157
	-	1000	99	23	25	23	162
	+	1	89	24	25	29	103
	+	10	81	22	38	11	117
	+	50	89	17	26	25	133
	+	100	128	11	25	27	134
	+	500	108	13	30	32	128
	+	1000	115	12	28	38	159

Table 27

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM*
OF ENDRIN

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		16	3	11	12	107
	+		8	5	18	10	134
Positive controls							
β-Propiolactone	-	50	328				666
2-Anthramine	-	10			3	18	
	+	10		173	1580	1416	
Endrin	-	10	11	5	14	20	102
	-	50	18	5	11	7	92
	-	100	19	8	12	13	107
	-	500	16	5	11	14	93
	-	1000	23	8	9	14	104
	-	5000	18	4	8	11	110
	+	10	12	6	15	28	89
	+	50	15	13	19	30	82
	+	100	7	8	15	30	106
	+	500	7	3	17	14	79
	+	1000	11	3	18	23	132
	+	5000	9	6	12	15	111

Table 28

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF ETHION

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		23	16	26	32	119
	+		8	18	19	35	124
Positive controls							
β-Propiolactone	-	50	894				
AF2	-	0.1					791
9-Aminoacridine	-	100		593			
2-Anthramine	-	2.5			12	24	
	+	2.5			120	161	
Ethion	-	50	29	7	20	25	104
	-	100	15	10	15	24	96
	-	500	29	8	18	31	106
	-	1000	25	7	14	22	94
	-	5000	20	7	22	26	118
	+	50	23	11	18	29	100
	+	100	9	15	27	33	94
	+	500	16	12	23	37	111
	+	1000	9	17	22	36	111
	+	5000	12	16	14	33	110

Table 29

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF ETHION

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		23	5	11	20	145
	+		27	7	28	30	191
Positive controls							
2-Anthramine	-	2.5			11	30	
	+	2.5			167	199	
9-Aminoacridine	-	100		388			
β-Propiolactone	-	50	447				
AF2	-	0.1					811
Ethion	-	50	28	9	8	7	137
	-	100	27	5	6	11	124
	-	500	20	4	10	18	145
	-	1000	31	3	10	16	160
	-	5000	21	4	9	14	159
	+	50	13	4	18	28	137
	+	100	16	9	23	25	160
	+	500	10	4	23	40	163
	+	1000	17	4	22	37	169
	+	5000	10	5	16	43	196

Table 30

IN VITRO ASSAYS WITH *SALMONELLA TYPHIMURIUM* OF ETHION

EXPERIMENT 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate TA100
Negative control	-		109
	+		113
Positive control 2-Anthramine	+	2.5	212
Ethion	-	1000	113
	-	2000	107
	-	3000	108
	-	4000	132
	-	5000	148
	+	1000	133
	+	2000	130
	+	3000	124
	+	4000	115
	+	5000	138

Table 31

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF FENSULFOTHION

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		42	23	37	76	84
	+		46	17	48	64	107
Positive control 2-Anthramine	-	20			64	76	
	+	20	198	70	2860	1740	1250
Fensulfothion	-	1	44	17	36	37	103
	-	10	42	21	48	50	95
	-	50	44	10	49	41	85
	-	100	42	15	56	43	105
	-	500	55	16	46	48	99
	-	1000	41	16	51	29	58
	+	1	40	22	58	65	110
	+	10	48	12	94	55	92
	+	50	41	16	65	57	90
	+	100	45	23	58	74	95
	+	500	46	19	53	57	77
	+	1000	44	15	55	60	100

Table 32

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF FENSULFOTHION
EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		14	18	14	22	121
	+		14	7	18	19	127
Positiive controls							
β-Propiolactone	-	50	633	365			617
2-Anthramine	-	50			18	38	
	+	50			2620	2956	
Fensulfothion	-	10	13	12	13	26	104
	-	50	18	14	14	14	120
	-	100	12	8	10	14	99
	-	500	16	16	12	20	102
	-	1000	10	14	19	27	107
	-	5000	13	13	11	20	82
	+	10	14	15	35	13	108
	+	50	14	14	29	24	95
	+	100	14	11	15	17	122
	+	500	21	18	15	26	101
	+	1000	27	18	15	20	88
	+	5000	12	12	13	25	105

Table 33

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF FONOFOS

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		17	12	15	20	144
	+		12	10	22	35	126
Positive controls							
2-Anthramine	-	20			21		
	+	20		180	1860	174	
N-Methyl-N'-nitro-N-nitrosoguanidine	-	2	366				562
Fonofos	-	1	15	12	10	83	131
	-	10	20	5	12	23	124
	-	50	26	8	13	18	112
	-	100	21	8	6	14	99
	-	500	22	8	12	13	117
	-	1000	33	2	3	16	99
	+	1	18	7	24	40	136
	+	10	19	8	17	24	156
	+	50	23	12	17	23	95
	+	100	21	8	20	24	97
	+	500	16	8	17	21	114
	+	1000	20	4	15	31	116

Table 34

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF FONOFOS

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		30	9	29	44	114
	+		20	17	32	39	152
Positive control 2-Anthramine	-	20				86	
	+	20	720	223	1080	1220	1200
Fonofos	-	10	32	15	28	30	135
	-	50	26	6	18	34	121
	-	100	29	16	15	21	118
	-	500	30	14	18	20	144
	-	1000	48	13	10	22	111
	-	5000	31	10	13	20	107
	+	10	13	15	13	46	124
	+	50	18	16	19	22	122
	+	100	18	22	19	30	146
	+	500	14	16	24	35	111
	+	1000	16	12	11	26	120
	+	5000	17	21	21	27	124

Table 35

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF METHOXYCHLOR

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		25	10	18	18	115
	+		14	6	26	30	108
Positive controls							
β-Propiolactone	-	50	922				
2-Anthramine	-	20			19	43	
	+	20		98	175	216	340
Methoxychlor	-	1	24	13	15	31	98
	-	10	29	5	26	30	106
	-	50	27	8	19	34	118
	-	100	41	8	17	38	113
	-	500	29	7	17	29	129
	-	1000	27	6	23	34	137
	+	1	13	8	15	29	137
	+	10	20	9	22	34	96
	+	50	12	5	22	19	88
	+	100	15	8	16	17	98
	+	500	16	8	27	32	99
	+	1000	15	8	24	24	115

Table 36

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF METHOXYCHLOR

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		38	13	20	23	149
	+		16	22	35	35	137
Positive controls							
2-Anthramine	-	20	53	19	24	29	190
	+	20	585	227	2087	295	2616
β-Propiolactone	-	50	1560				1120
Methoxychlor	-	50	42	16	12	16	183
	-	100	68	16	23	16	145
	-	500	56	12	16	27	167
	-	1000	58	10	14	20	168
	-	2500	66	17	14	17	172
	-	5000	50	17	31	24	170
	+	50	22	10	22	45	164
	+	100	22	11	45	31	150
	+	500	16	9	22	36	151
	+	1000	18	12	33	30	150
	+	2500	27	26	35	28	155
	+	5000	24	19	33	46	160

Table 37

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF PROPANIL

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		19	5	12	22	157
	+		20	8	20	31	104
Positive controls							
β-Propiolactone	-	50	280	375			375
2-Anthramine	-	10			14	32	
	+	10			1545	1661	
Propanil	-	10	12	1	8	17	88
	-	50	20	6	8	18	95
	-	100	9	6	8	24	120
	-	500	11	4	10	20	60
	-	1000	T*	T	T	T	68
	-	5000	T	T	T	T	T
	+	10	9	9	11	21	107
	+	50	12	9	7	26	84
	+	100	8	11	7	26	90
	+	500	13	8	23	45	54
	+	1000	T	T	T	56	68
	+	5000	T	T	T	T	T

---

* T , toxic.

Table 38

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF PROPANIL

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		5	11	15	26	171
	+		6	15	24	40	193
Positive controls							
β-Propiolactone	-	50	85				288
2-Anthramine	-	10			6	26	
	+	10		167	1224	847	
Propanil	-	10	9	7	5	22	143
	-	50	14	11	13	18	154
	-	100	12	12	5	24	137
	-	250	7	7	6	14	99
	-	500	4	7	6	17	75
	-	1000	5	T*	T	T	T
	+	10	5	8	18	28	143
	+	50	8	7	24	33	149
	+	100	6	9	21	34	147
	+	250	7	9	25	30	135
	+	500	5	10	37	46	121
	+	1000	T	T	T	T	T

---

* T , toxic.

Table 39

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF PROPANIL

EXPERIMENT 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate TA100
Negative control	-		109
	+		113
Positive control 2-Anthramine	+	2.5	212
Propanil	-	10	101
	-	50	102
	-	100	107
	-	500	80
	-	1000	50
	+	10	132
	+	50	108
	+	100	131
	+	500	91
	+	1000	46

Table 40

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF SIDURON

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		42	23	37	76	84
	+		46	17	48	64	107
Positive control 2-Anthramine	-	20			64	76	
	+	20	198	370	2860	1740	1250
Siduron	-	1	49	11	59	43	98
	-	10	58	15	58	44	104
	-	50	43	23	59	42	71
	-	100	59	9	62	33	72
	-	500	65	13	46	20	58
	-	1000	57	20	29	25	83
	+	1	32	20	60	33	118
	+	10	38	20	70	42	102
	+	50	43	25	44	39	77
	+	100	27	20	56	35	86
	+	500	41	25	54	24	6
	+	1000	41	20	61	41	T*

---

* T, toxic.

Table 41

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF SIDURON
EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		29	15	30	44	109
	+		25	18	33	51	113
Positive control							
AF2	-	0.5				190	
2-Anthramine	+	20	1330	700	3900	4940	212
Siduron	-	10	31	21	30	42	101
	-	50	30	14	33	51	129
	-	100	32	23	28	56	101
	-	500	33	17	22	43	121
	-	1000	36	15	25	27	118
	-	5000	27	13	12	39	57
	+	10	35	23	47	42	110
	+	50	27	24	30	46	105
	+	100	30	27	42	66	95
	+	500	28	19	28	59	119
	+	1000	29	13	41	40	81
	+	5000	31	15	37	39	21

Table 42

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM

OF TRICHLORFON

EXPERIMENT 1

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		17	5	8	28	118
	+		10	4	20	39	133
Positive controls							
2-Anthramine	+	20		175	1380	2400	
N-Methyl-N'-nitro-N-nitrosoguanidine	-	2	2820				1800
Trichlorfon	-	1	10	4	13	22	103
	-	10	6	4	13	21	99
	-	50	8	5	10	27	101
	-	100	8	6	15	34	106
	-	500	12	3	9	31	120
	-	1000	7	4	6	23	134
	+	1	8	6	19	41	135
	+	10	13	10	10	35	102
	+	50	8	10	22	59	114
	+	100	9	6	11	34	122
	+	500	9	5	13	34	121
	+	1000	11	5	19	41	154

Table 43

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF TRICHLORFON

EXPERIMENT 2

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate				
			TA1535	TA1537	TA1538	TA98	TA100
Negative control	-		30	13	25	20	131
	+		17	14	17	35	124
Positive control							
N-Methyl-N'-nitro-N-nitrosoguanidine	-	2	186				494
2-Anthramine	-	20			27		
	+	20		150	2400	174	
Trichlorfon	-	10	18	9	7	27	136
	-	50	16	7	11	22	159
	-	100	17	14	12	40	135
	-	500	15	12	10	15	141
	-	1000	13	14	7	24	173
	-	5000	14	12	14	16	186
	+	10	10	12	21	23	145
	+	50	15	10	19	23	143
	+	100	11	12	11	19	129
	+	500	9	10	23	34	151
	+	1000	13	13	19	31	151
	+	5000	12	11	18	15	205

Table 44

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM OF TRICHLORFON

EXPERIMENT 3

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Histidine Revertants per Plate
			TA100
Negative control	-		115
	+		108
Positive control 2-Anthramine	+	2.5	340
Trichlorfon	-	500	118
	-	1000	115
	-	2500	135
	-	5000	637
	-	7500	651
	-	10000	269
	+	500	117
	+	1000	137
	+	2500	155
	+	5000	181
	+	7500	249
	+	10000	274

Table 45

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2
OF
ACEPHATE, ASPON, CARBOFURAN, CROTOZYPHOS, DEMETON, DIAZINON, DICAMBA, DISULFOTON, AND ETHION

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Tryptophan Revertants per Plate								
			Acephate	Aspon	Carbofuran	Crotoxyphos	Demeton	Diazinon	Dicamba	Disulfoton	Ethion
Negative control	-		40	40	25	25	25	24	40	26	30
	+		51	51	37	37	37	49	51	49	27
Pocitive controls											
2-Anthramine	+	2.5	720	720					720		
β-Propiolactone	-	10						91		91	
N-Methyl-N'-nitro-N-nitrosoguanidine	-	2.0			191	191	191				
AF2	-	0.1									147
Experiment 1	-	1	44	56	51	39	40	33	Contam.	21	
	-	10	45	51	56	33	40	22	41	23	25
	-	50	50	31	56	27	61	30	30	21	20
	-	100	60	46	44	30	77	33	28	24	25
	-	500	38	36	56	35	132	26	30	17	19
	-	1000	59	62	53	39	237	25	46	28	22
	+	1	45	40	39	55	57	24	54	31	28
	+	10	51	57	61	36	70	25	54	21	27
	+	50	58	54	63	34	105	29	53	41	25
	+	100	51	50	60	40	148	25	51	27	31
	+	500	46	52	66	38	351	21	Contam.	26	34
	+	1000	58	66	50	56	652	31	54	22	30

Table 45 (concluded)

Compound	Metabolic Activation	Micrograms of Compound Added per Plate	Tryptophan Revertants per Plate								
			Acephate	Aspon	Carbofuran	Cytoxyphos	Demeton	Diazinon	Dicamba	Disulfoton	Ethion
Negative control	-		27	28	40	31	55	59	15	31	35
	+		20	30	31	37	58	59	35	37	36
Positive controls											
N-Methyl-N'-nitro-N-nitrosoguanidine	-	2.0						270			
AF-2	-	0.1		244		1044				1044	1202
2-Anthramine	-	2.5			41				37		
	+	2.5			720		326		1048		
Experiment 2	-	10	NT*	28	53	37	84	72	30	34	39
	-	50	NT	22	40	42	135	45	30	35	27
	-	100	NT	26	58	36	168	41	29	32	26
	-	500	41	36	43	36	264	54	31	29	29
	-	1000	27	30	47	28	442	41	22	45	26
	-	5000	42	30	38	28	538	46	0	24	1
	-	10000	52	NT	NT	NT	NT	NT	NT	NT	NT
	+	10	NT	21	63	42	82	51	21	33	47
	+	50	NT	21	43	41	166	61	24	32	27
	+	100	NT	27	48	30	216	57	30	31	36
	+	500	27	30	40	34	406	58	27	38	35
	+	1000	30	26	33	22	518	66	20	45	31
	+	5000	40	27	31	40	948	52	14	42	8
	+	10000	59	NT	NT	NT	NT	NT	NT	NT	NT

---

* NT, not tested.

Table 46

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2 - ACEPHATE

Controls	Metabolic Activation	Micrograms of Compound Added per Plate	Tryptophan Revertants per Plate Experiment 3	Tryptophan Revertants per Plate Experiment 4
Negative control	-		25	24
	+		37	46
Positive control AF2	-	0.1	158	131
Acephate	-	1000	34	NT*
	-	2500	25	37
	-	5000	24	33
	-	6000	24	49
	-	8000	42	47
	-	10000	34	38
	+	1000	26	NT
	+	2500	38	47
	+	5000	45	36
	+	6000	37	45
	+	8000	27	44
	+	10000	48	67

---

* NT, not tested.

Table 47

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2
ON
FENSULFOTHION, FONOFOS, METHOXYCHLOR, PROPANIL, SIDURON, TRICHLORFON, 2,4-D ACID, 2,4-DB ACID, AND ENDRIN

Controls	Metabolic Activation	Micrograms of Compound Added per Plate	Tryptophan Revertants per Plate								
			Fensulfothion	Fonofos	Methoxychlor	Propanil	Siduron	Trichlorfon	2,4-D	2,4-DB	Endrin
Negative control	-		84	25	23	23	23	40	23	34	24
			62	37	26	26	26	51	26	24	47
Positive controls 2-Anthramine	+	2.5	1380	191	370	370	370	720	370	320	573
Experiment 1	-	1	56	59	19	20	27	37	32	23	21
	-	10	78	57	38	28	35	55	27	20	24
	-	50	61	42	20	24	33	47	35	12	20
	-	100	62	48	33	23	23	57	22	19	26
	-	500	51	54	18	25	28	68	21	18	26
	-	1000	46	45	23	17	25	93	10	Toxic	27
	+	1	72	66	30	30	33	44	28	14	17
	+	10	77	62	29	26	35	45	26	14	24
	+	50	60	59	22	32	33	73	21	28	30
	+	100	77	51	19	29	31	48	23	26	30
	+	500	75	51	34	27	39	62	25	45	33
	+	1000	62	47	28	20	40	99	16	15	23

---

* NT, not tested.

Table 47 (concluded)

Controls	Metabolic Activation	Micrograms of Compound Added per Plate	Tryptophan Revertants per Plate: Fensulfothion	Fonofos	Methoxychlor	Propanil	Siduron	Trichlorfon	2,4-D	2,4-DB	Endrin
Negative control	-		40	31	45	34	84	59	34	35	26
	+		51	37	48	24	62	59	24	36	45
Positive controls											
AF2	-	0.1		1044						1202	441
2-Anthramine	-	2.5	41								
	+	2.5	720								
β-Propiolactone	-	10.0				320	1380		320		
N-Methyl-N'-nitro-N-nitrosoguanidine	-	2.0						270			
Experiment 2	-	10	51	44	28	28	55	64	20	30	34
	-	50	52	35	32	18	63	49	23	29	41
	-	100	46	37	25	18	67	62	31	28	42
	-	500	33	27	41	16	61	59	21	22	32
	-	1000	43	46	34	14	46	74	26	24	15
	-	5000	56	32	28	Toxic	38	112	Toxic	Toxic	15
	+	10	46	27	48	18	82	55	10	41	39
	+	50	45	35	31	15	76	57	23	34	38
	+	100	62	42	48	23	73	60	18	28	47
	+	500	46	27	34	12	60	63	18	21	47
	+	1000	66	33	53	17	54	66	22	27	36
	+	5000	54	33	Contam.	Toxic	46	142	22	20	43

Table 48

IN VITRO ASSAYS WITH ESCHERICHIA COLI WP2 - TRICHLORFON

Controls	Metabolic Activation	Micrograms of Compound Added per Plate	Tryptophan Revertants per Plate Experiment 3
Negative control	-		28
	+		30
Positive control AF2	-	0.5	244
Trichlorfon	-	500	23
	-	1000	32
	-	2500	31
	-	5000	43
	-	7500	76
	-	10000	44
	+	500	30
	+	1000	32
	+	2500	43
	+	5000	65
	+	7500	58
	+	10000	80

Table 49

DIFFERENTIAL TOXICITY OF REPAIR-PROFICIENT AND -DEFICIENT MICROORGANISMS

Compound	mg of Compound in 10 μl of DMSO Applied to Disc	Diameter of Zone of Inhibition (mm)*			
		B. subtilis		E. coli	
		H17	M45	W3110	p3478
Negative control Chloramphenicol	20 μg	38	40	40	40
Positive control 1-Phenyl-3,3-dimethyl-triazene	2	12	20	13	20
Acephate	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6
Aspon	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6
Carbofuran	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6
Crotoxyphos	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6
Diazinon	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6

---

* The diameter of the disc was 6mm.

Table 49 (continued)

Compound	mg of Compound in 10 1 of DMSO Applied to Disc	Diameter of Zone of Inhibition (mm)			
		B. subtilis		E. coli	
		H17	M45	W3110	p3478
Dicamba	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	7	6	7
	5.0	10	14	10	13
Disulfoton	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6
Fensulfothion	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6
Fonofos	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6
Methoxychlor	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6
Siduron	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6
Trichlorfon	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6

Table 49 (concluded)

Compound	mg of Compound in 10 µl of DMSO Applied to Disc	Diameter of Zone of Inhibition (mm)			
		B. subtilis		E. coli	
		H17	M45	W3110	p3478
Negative control Chloramphenicol	20 µg	24	23	26	27
Positive control 1-Phenyl-3,3-dimethyl-triazene	2	34	64	36	65
2,4-D acid	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	11	10	11	10
	5.0	15	20	16	20
2,4-DB acid	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	8	6	6
	5.0	6	13	6	7
Demeton	1.0	6	6	6	6
	5.0	6	10	6	6
	10.0	7	12	7	7
Ethion	0.01	6	6	6	6
	0.10	6	6	6	6
	1.0	6	6	6	6
	5.0	6	6	6	6
Propanil	0.01	6	6	6	6
	0.10	7	12	6	6
	1.0	8	15	8	8
	5.0	8	16	9	9

Table 50

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ACEPHATE

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml ($\times 10^{-7}$)	Survivors: Percent	Mitotic Recombinants: Per ml ($\times 10^{-3}$)	Mitotic Recombinants: Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		5.3	100	4	7.5
	+		5.3	100	4	7.5
Acephate	-	0.1	6.3	119	4	6.3
	-	0.5	5.7	108	6	10.5
	-	1.0	5.1	96	5	9.8
	-	5.0	4.7	89	46	98.0
	+	0.1	5.4	102	1	1.9
	+	0.5	5.6	106	7	12.5
	+	1.0	5.4	102	4	7.4
	+	5.0	4.5	84	38	84.4
		EXPERIMENT 2				
Negative control	-		6.0	100	2.5	4.2
	+		5.6	100	4	7.1
Acephate	-	1.0	6.5	108	7	10.8
	-	2.0	6.0	100	19	31.7
	-	4.0	5.3	88	33	62.3
	-	5.0	5.3	88	44	83.0
	+	1.0	5.4	96	11	20.4
	+	2.0	5.5	98	8	14.5
	+	4.0	6.2	111	35	56.4
	+	5.0	5.5	98	51	92.7

Table 51

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ASPON

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml (x 10^{-7})	Survivors: Percent	Mitotic Recombinants: Per ml (x 10^{-3})	Mitotic Recombinants: Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		7.0	100	4	5.7
	+		6.4	100	2	6.3
Positive control 1,2,3,4-Diepoxybutane	-	0.04	4.9	70	987	2014
	+	0.04	5.8	91	1090	1879
Aspon	-	0.1	6.4	91	6	9.4
	-	0.5	5.6	80	6	10.7
	-	1.0	7.3	104	4	5.5
	-	5.0	6.9	99	3	4.3
	+	0.1	5.9	92	2	3.4
	+	0.5	6.9	108	4	5.8
	+	1.0	8.0	125	12	15.0
	+	5.0	6.9	108	7	10.1
		EXPERIMENT 2				
Negative control	-		5.3	100	4	7.5
	+		5.3	100	4	7.5
Aspon	-	1.0	5.2	98	3	5.8
	-	2.0	4.8	91	3	6.3
	-	4.0	5.8	109	1	1.7
	-	5.0	5.6	106	2	3.6
	+	1.0	5.2	98	5	9.6
	+	2.0	5.3	100	3	5.7
	+	4.0	5.0	94	6	12.0
	+	5.0	5.2	98	4	7.7

Table 52

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
CARBOFURAN

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml ($\times 10^{-7}$)	Survivors: Percent	Mitotic Recombinants: Per ml ($\times 10^{-3}$)	Mitotic Recombinants: Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		9.0	100	6	6.7
	+		7.9	100	5	6.3
Positive control	-	0.04	7.6	84	1125	1480
1,2,3,4-Diepoxybutane	+	0.04	6.7	85	965	1440
Carbofuran	-	0.1	8.2	91	8	9.8
	-	0.5	8.3	92	9	10.8
	-	1.0	8.3	92	2	2.4
	-	5.0	9.7	86	11	14.3
	+	0.1	6.4	81	6	9.4
	+	0.5	7.4	94	8	10.8
	+	1.0	6.8	86	2	2.9
	+	5.0	7.0	89	8	11.4
		EXPERIMENT 2				
Negative control	-		4.4	100	5	11.4
	+		4.9	100	5	10.2
Positive control	-	0.04	3.3	75	715	2167
1,2,3,4-Diepoxybutane	+	0.04	3.7	76	1015	2743
Carbofuran	-	1.0	3.9	89	8	20.5
	-	2.0	2.4	54	4	16.7
	-	4.0	1.9	43	11	57.9
	-	5.0	1.4	32	4	28.6
	+	1.0	4.9	100	10	20.4
	+	2.0	4.0	82	5	12.5
	+	4.0	2.2	45	8	36.4
	+	5.0	1.6	33	1	6.3

Table 53

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
CROTOXYPHOS

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		9.0	100	6	6.7
	+		7.9	100	5	6.3
Positive control	-	0.04	7.6	84	1125	1480
1,2,3,4-Diepoxybutane	+	0.04	6.7	85	965	1440
Crotoxyphos	-	0.1	7.1	79	4	5.6
	-	0.5	6.8	76	14	20.6
	-	1.0	5.7	63	11	19.3
	-	5.0	7.2	80	19	26.3
	+	0.1	6.5	82	3	4.6
	+	0.5	5.0	63	15	30.0
	+	1.0	5.6	71	21	37.5
	+	5.0	4.8	61	11	22.9
		EXPERIMENT 2				
Negative control	-		6.2	100	2	3.2
	+		5.1	100	4	6.6
Crotoxyphos	-	1.0	3.4	55	9	2.6
	-	2.0	2.7	44	3	11.1
	-	4.0	3.9	63	11	32.4
	-	5.0	4.0	65	16	40.0
	+	1.0	3.1	51	5	1.6
	+	2.0	3.0	49	7	23.3
	+	4.0	4.0	66	10	25.0
	+	5.0	4.0	66	9	22.5

Table 54

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
2,4-D ACID

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^{5} Survivors
		EXPERIMENT 1				
Negative control	-		6.1	100	6	9.8
	+		5.2	100	5	9.6
Positive control	-	0.04	5.5	90	1135	2063
1,2,3,4-Diepoxybutane	+	0.04	5.4	104	1140	2111
2,4-D acid	-	0.1	5.1	84	5	9.8
	-	0.5	4.5	74	4	8.9
	-	1.0	T*			
	+	0.1	5.6	108	5	8.9
	+	0.5	4.5	87	6	13.3
	+	1.0	T*			
		EXPERIMENT 2				
Negative control	-		5.3	100	3	5.7
	+		5.4	100	3	5.6
Positive control	-	0.04	2.6	49	827	3181
1,2,3,4-Diepoxybutane	+	0.04	4.0	74	1002	2505
2,4-D acid	-	0.10	4.9	92	1	2.0
	-	0.25	5.3	100	4	7.6
	-	0.50	4.2	79	5	11.9
	-	0.75	3.1	58	5	16.1
	+	0.10	5.1	94	1	2.0
	+	0.25	5.8	107	7	12.1
	+	0.50	3.7	69	4	10.8
	+	0.75	3.3	61	1	3.0

---

* T, toxic.

Table 55

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
2,4-DB ACID

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors Cells per ml (x 10^{-7})	Survivors Percent	Mitotic Recombinants Per ml (x 10^{-3})	Mitotic Recombinants Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		6.1	100	6	9.8
	+		5.2	100	5	9.6
Positive control	-	0.04	5.5	90	1135	2063
1,2,3,4-Diepoxybutane	+	0.04	5.4	104	1140	2111
2,4-DB acid	-	0.1	5.6	92	6	10.7
	-	0.5	4.6	75	6	13.0
	-	1.0	2.1	34	2	9.5
	-	5.0	1.7	28	3	17.7
	+	0.1	5.8	112	1	1.7
	+	0.5	4.2	81	5	11.9
	+	1.0	0.8	2	1	12.5
	+	5.0	T*			
		EXPERIMENT 2				
Negative control	-		5.3	100	3	5.7
	+		5.4	100	3	5.6
Positive control	-	0.04	2.6	49	827	3181
1,2,3,4-Diepoxybutane	+	0.04	4.0	74	1002	2505
2,4-DB acid	-	0.25	5.0	94	3	6.0
	-	0.50	4.6	87	4	8.7
	-	0.75	3.0	57	5	16.7
	-	1.0	1.2	23	2	16.7
	+	0.25	5.3	98	2	3.8
	+	0.50	3.8	70	2	5.3
	+	0.75	2.5	46	6	24.0
	+	1.0	1.7	31	1	17.0

---

* T , toxic.

Table 56

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
DEMETON

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml $(\times 10^{-7})$	Survivors: Percent	Mitotic Recombinants: Per ml $(\times 10^{-3})$	Mitotic Recombinants: Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		6.5	100	4	6.2
	+		6.4	100	2	3.1
Positive control 1,2,3,4-Diepoxybutane	-	0.04	6.2	95	1235	1992
	+	0.04	5.8	91	835	1440
Demeton	-	0.1	6.0	92	171	285
	-	0.5	5.9	91	155	263
	-	1.0	5.0	77	117	234
	-	5.0	3.5	54	93	266
	+	0.1	5.3	83	70	132
	+	0.5	4.5	70	201	447
	+	1.0	4.5	70	125	278
	+	5.0	3.3	52	88	267
		EXPERIMENT 2				
Negative control	-		4.8	100	3	6.3
	+		4.7	100	3	6.3
Positive control 1,2,3,4-Diepoxybutane	-	0.04	3.4	71	745	2191
	+	0.04	3.5	74	683	1951
Demeton	-	1.0	1.7	35	79	465
	-	2.0	0.5	10	33	660
	-	4.0	1.0	21	28	280
	-	5.0	1.4	29	33	236
	+	1.0	1.6	34	57	356
	+	2.0	0.9	19	17	189
	+	4.0	1.1	23	28	254
	+	5.0	1.1	23	33	300

Table 56 (concluded)

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml (x 10^{-7})	Survivors: Percent	Mitotic Recombinants: Per ml (x 10^{-3})	Mitotic Recombinants: Per 10^5 Survivors
		EXPERIMENT 3				
Negative control	-		6.8	100	6	8.2
	+		6.2	100	3	6.5
Positive control 1,2,3,4-Diepoxybutane	-	0.04	7.5	110	392	523
	+	0.04	7.2	116	411	571
Demeton	-	0.5	6.6	97	124	188
	-	1.0	5.4	79	98	181
	-	2.5	4.3	63	87	202
	-	5.0	4.7	69	44	94
	+	0.5	5.0	81	121	242
	+	1.0	5.6	90	79	141
	+	2.5	4.1	66	60	146
	+	5.0	4.8	71	44	92

Table 57

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
DIAZINON

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		6.4	100	3	4.7
	+		5.8	100	3	5.2
Positive control 1,2,3,4-Diepoxybutane	-	0.04	4.4	69	960	2181
	+	0.04	3.8	66	672	1768
Diazinon	-	0.1	5.0	78	1	2.0
	-	0.5	4.6	72	3	6.5
	-	1.0	5.0	78	1	2.0
	-	5.0	5.3	83	4	7.5
	+	0.1	4.6	79	1	2.2
	+	0.5	4.0	69	1	2.5
	+	1.0	4.0	69	2	5.0
	+	5.0	4.3	74	4	9.3
		EXPERIMENT 2				
Negative control	-		4.4	100	5	11.4
	+		4.9	100	5	10.2
Positive control 1,2,3,4-Diepoxybutane	-	0.04	3.3	75	715	2167
	+	0.04	3.7	76	1015	2743
Diazinon	-	1.0	3.9	89	4	10.3
	-	2.0	3.5	80	5	14.3
	-	4.0	4.0	91	6	15.0
	-	5.0	3.8	86	9	23.7
	+	1.0	3.1	63	4	12.9
	+	2.0	3.3	67	5	15.2
	+	4.0	3.9	80	7	17.9
	+	5.0	3.8	78	12	31.6

Table 57(concluded)

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^{5} Survivors
		EXPERIMENT 3				
Negative control	-		8.2	100	2	2.4
	+		8.1	100	3	3.7
Positive control	-	0.04	6.1	74	868	1423
1,2,3,4-Diepoxybutane	+	0.04	5.7	70	945	1658
Diazinon	-	1.0	5.7	70	8	14.0
	-	3.0	7.7	94	3	3.9
	-	4.0	7.3	89	2	2.7
	-	5.0	7.4	90	3	4.1
	+	1.0	6.1	75	1	1.6
	+	3.0	6.3	78	3	4.8
	+	4.0	6.8	84	2	2.9
	+	5.0	7.0	86	2	2.9

Table 58

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
DICAMBA

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		4.2	100	4.5	10.7
	+		4.6	100	2.5	5.4
Dicamba	-	0.1	5.6	133	5	8.9
	-	0.5	3.8	90	1	2.6
	-	1.0	1.4	33	1	7.1
	-	5.0	T*			
	+	0.1	4.4	96	2	4.5
	+	0.5	3.0	65	2	6.7
	+	1.0	2.0	43	2	10.0
	+	5.0	T			
		EXPERIMENT 2				
Negative control	-		6.0	100	2.5	4.2
	+		5.6	100	4	7.1
Dicamba	-	0.10	5.6	93	3	5.4
	-	0.25	6.4	107	9	14.1
	-	0.50	4.8	80	1	2.1
	-	1.0	1.2	20	1	8.3
	+	0.10	5.0	89	5	10.0
	+	0.25	6.5	116	8	12.3
	+	0.50	4.5	80	3	6.7
	+	1.0	3.8	68	1	3.8

Table 59

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
DISULFOTON

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		7.8	100	2	2.6
	+		7.0	100	4	5.7
Positive control	-	0.04	6.6	85	1602	2427
1,2,3,4-Diepoxybutane	+	0.04	5.6	80	1365	2437
Disulfoton	-	0.1	8.3	106	6	7.2
	-	0.5	7.2	92	3	4.2
	-	1.0	9.4	120	12	12.8
	-	5.0	9.3	119	5	5.4
	+	0.1	8.0	114	3	4.2
	+	0.5	8.1	116	5	6.2
	+	1.0	9.4	134	8	8.5
	+	5.0	7.9	113	14	17.7
		EXPERIMENT 2				
Negative control	-		4.8	100	3	6.3
	+		4.7	100	3	6.4
Positive control	-	0.04	3.4	71	745	2191
1,2,3,4-Diepoxybutane	+	0.04	3.5	74	683	1951
Disulfoton	-	1.0	3.5	73	2	5.7
	-	2.0	4.3	90	4	9.3
	-	4.0	4.5	94	4	8.9
	-	5.0	5.4	113	3	5.6
	+	1.0	3.9	83	3	7.7
	+	2.0	4.3	91	8	18.6
	+	4.0	4.7	100	5	10.6
	+	5.0	4.6	98	9	19.6

Table 59 (concluded)

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml ($\times 10^{-7}$)	Survivors: Percent	Mitotic Recombinants: Per ml ($\times 10^{-3}$)	Mitotic Recombinants: Per 10^5 Survivors
		EXPERIMENT 3				
Negative control	-		8.2	100	2	2.4
	+		8.1	100	3	3.7
Positive control	-	0.04	6.1	74	868	1423
1,2,3,4-Diepoxybutane	+	0.04	5.7	70	945	1658
Disulfoton	-	1.0	9.1	111	2	2.2
	-	2.0	7.3	89	3	4.1
	-	4.0	5.9	72	9	15.3
	-	5.0	8.5	104	4	4.7
	+	1.0	8.6	106	4	4.7
	+	2.0	7.7	95	7	9.1
	+	4.0	7.9	98	2	2.5
	+	5.0	7.8	96	6	7.7

Table 60

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ENDRIN

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml (x 10^{-7})	Survivors: Percent	Mitotic Recombinants: Per ml (x 10^{-3})	Mitotic Recombinants: Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		6.8	100	7	10.3
	+		5.7	100	5	8.8
Positive control 1,2,3,4-Diepoxybutane	-	0.04	3.2	47	687	2147
Endrin	-	0.1	4.5	66	4	8.9
	-	0.5	3.5	51	4	11.4
	-	1.0	4.5	66	6	13.3
	-	5.0	5.0	74	5	10.5
	+	0.1	3.4	60	9	26.5
	+	0.5	4.1	72	5	12.2
	+	1.0	3.9	68	3	7.7
	+	5.0	3.8	67	7	18.4
		EXPERIMENT 2				
Negative control	-		6.8	100	5	7.3
	+		6.1	100	3	4.9
Positive control 1,2,3,4-Diepoxybutane	-	0.04	4.8	71	1015	2114
Endrin	-	1.0	7.5	110	3	4.0
	-	2.0	6.9	101	2	2.9
	-	3.0	7.1	104	3	4.2
	-	5.0	5.9	87	3	5.1
	+	1.0	6.6	108	3	4.6
	+	2.0	6.9	113	2	2.9
	+	3.0	6.6	108	3	4.5
	+	5.0	6.5	106	3	4.6

Table 61

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
ETHION

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml (x 10^{-7})	Survivors: Percent	Mitotic Recombinants: Per ml (x 10^{-3})	Mitotic Recombinants: Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		5.5	100	4	7.3
	+		5.2	100	5	9.6
Positive control	-	0.04	4.5	82	1207	2682
1,2,3,4-Diepoxybutane	+	0.04	4.8	92	1320	2750
Ethion	-	0.1	5.9	107	2	3.4
	-	0.5	6.0	109	2	3.3
	-	1.0	4.0	73	6	15.0
	-	5.0	5.3	96	3	5.7
	+	0.1	5.6	108	7	12.5
	+	0.5	4.8	92	4	8.3
	+	1.0	4.9	94	2	4.1
	+	5.0	4.9	94	1	2.0
		EXPERIMENT 2				
Negative control	-		3.1	100	1	3.2
	+		2.8	100	1	3.6
Positive control	-	0.04	4.5	82	1207	2682
1,2,3,4-Diepoxybutane	+	0.04	4.8	92	1320	2750
Ethion	-	0.1	5.9	107	2	3.4
	-	0.5	6.0	109	2	3.3
	-	1.0	4.0	73	6	15.0
	-	5.0	5.3	96	3	5.7
	+	0.1	5.6	108	7	12.5
	+	0.5	4.8	92	4	8.3
	+	1.0	4.9	94	2	4.1
	+	5.0	4.9	94	1	2.0

Table 62

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
FENSULFOTHION

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		7.0	100	4	5.7
	+		6.4	100	2	6.3
Positive control 1,2,3,4-Diepoxybutane	-	0.04	4.9	70	987	2014
	+	0.04	5.8	91	1090	1879
Fensulfothion	-	0.1	6.0	86	5	8.3
	-	0.5	1.7	24	1	5.9
	-	1.0	2.0	29	1	5.0
	-	5.0	2.5	36	3	12.0
	+	0.1	4.7	91	4	8.5
	+	0.5	2.2	34	4	18.2
	+	1.0	2.2	34	2	9.1
	+	5.0	2.2	34	1	4.5
		EXPERIMENT 2				
Negative control	-		6.2	100	2	3.2
	+		6.1	100	4	6.6
Fensulfothion	-	0.10	4.4	71	4	9.1
	-	0.25	3.5	56	6	17.1
	-	0.50	2.4	39	1	4.2
	-	1.0	1.9	31	2	10.5
	+	0.10	4.5	73	7	15.6
	+	0.25	3.9	64	3	7.7
	+	0.50	2.2	36	4	18.2
	+	1.0	1.3	21	2	15.4

Table 63

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3

FONOFOS

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml ($x\ 10^{-7}$)	Survivors: Percent	Mitotic Recombinants: Per ml ($x\ 10^{-3}$)	Mitotic Recombinants: Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		6.2	100	1	1.6
	+		4.9	100	2	4.1
Positive control	-	0.04	5.0	81	1397	2794
1,2,3,4-Diepoxybutane	+	0.04	4.5	92	820	1822
Fonofos	-	0.1	5.9	95	3	5.1
	-	0.5	6.5	105	8	12.3
	-	1.0	5.8	94	3	5.2
	-	5.0	5.5	89	3	5.5
	+	0.1	5.0	102	5	10.0
	+	0.5	5.2	106	2	3.8
	+	1.0	4.3	88	2	4.7
	+	5.0	5.2	106	3	5.8
		EXPERIMENT 2				
Negative control	-		4.4	100	5	11.4
	+		4.9	100	5	10.2
Positive control	-	0.04	3.3	75	715	2167
1,2,3,4-Diepoxybutane	+	0.04	3.7	76	1015	2743
Fonofos	-	1.0	3.2	73	3	9.4
	-	2.0	2.6	59	4	15.4
	-	4.0	3.4	77	8	23.5
	-	5.0	2.7	61	8	29.6
	+	1.0	3.8	78	7	18.4
	+	2.0	3.0	61	3	10.0
	+	4.0	3.9	80	5	12.8
	+	5.0	3.0	61	4	13.3

Table 64

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
METHOXYCHLOR

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		6.8	100	7	10.3
	+		5.7	100	5	8.8
Positive control 1,2,3,4-Diepoxybutane	-	0.04	3.2	47	687	2147
Methoxychlor	-	0.1	3.7	54	7	18.9
	-	0.5	4.2	62	10	23.8
	-	1.0	4.6	68	4	8.7
	-	5.0	6.2	91	7	11.3
	+	0.1	4.7	82	4	8.5
	+	0.5	3.6	63	3	8.3
	+	1.0	5.1	89	5	9.8
	+	5.0	2.8	49	3	10.7
		EXPERIMENT 2				
Negative control	-		6.8	100	5	7.3
	+		6.1	100	3	4.9
Positive control 1,2,3,4-Diepoxybutane	-	0.04	4.8	71	1015	2114
Methoxychlor	-	1.0	5.5	81	3	5.5
	-	2.0	7.0	103	3	4.3
	-	4.0	7.1	104	3	4.2
	-	5.0	6.1	90	1	1.6
	+	1.0	5.9	97	2	3.4
	+	2.0	6.3	103	3	4.8
	+	4.0	6.1	100	5	8.2
	+	5.0	6.9	113	2	2.9

Table 65

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
PROPANIL

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml ($x\ 10^{-7}$)	Percent	Per ml ($x\ 10^{-3}$)	Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		6.1	100	6	9.8
	+		5.2	100	5	9.6
Positive control	-	0.04	5.5	90	1135	2063
1,2,3,4-Diepoxybutane	+	0.04	5.4	104	1140	2111
Propanil (Stam Tech)	-	0.1	2.0	33	6	30
	-	0.5	1.1	18	1	9.1
	-	1.0	0.2	0.3	T*	
	-	5.0	T*			
	+	0.1	2.3	44	5	22
	+	0.5	1.3	25	1	7.7
	+	1.0	0.8	2	T*	
	+	5.0	T*			
		EXPERIMENT 2				
Negative control	-		5.3	100	3	5.7
	+		5.4	100	3	5.6
Positive control	-	0.04	2.6	49	827	3181
1,2,3,4-Diepoxybutane	+	0.04	4.0	74	1002	2505
Propanil (Stam Tech)	-	0.010	6.3	119	2	3.2
	-	0.025	5.5	104	2	3.6
	-	0.050	5.8	109	6	10.3
	-	0.10	4.6	87	6	13.0
	+	0.010	5.1	94	5	9.8
	+	0.025	4.3	80	3	7.0
	+	0.050	5.8	107	2	3.5
	+	0.10	4.4	81	3	6.8

---

* T - Toxic

Table 66

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
SIDURON

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors: Cells per ml ($x\ 10^{-7}$)	Survivors: Percent	Mitotic Recombinants: Per ml ($x\ 10^{-3}$)	Mitotic Recombinants: Per 10^{5} Survivors
		EXPERIMENT 1				
Negative control	-		7.8	100	2	2.6
	+		7.0	100	4	5.7
Positive control	-	0.04	6.6	85	1602	2427
1,2,3,4-Diepoxybutane	+	0.04	5.6	80	1365	2437
Siduron	-	0.1	9.2	118	7	7.6
	-	0.5	8.2	105	7	8.5
	-	1.0	7.3	94	8	11.0
	-	5.0	9.2	118	5	5.4
	+	0.1	7.7	110	4	7.7
	+	0.5	7.8	111	6	7.7
	+	1.0	8.1	116	6	7.4
	+	5.0	7.1	101	3	4.2
		EXPERIMENT 2				
Negative control	-		4.5	100	5	11.1
	+		4.2	100	3	7.1
Positive control	-	0.04	4.5	100	870	1933
1,2,3,4-Diepoxybutane	+	0.04	5.2	100	653	1555
Siduron	-	1.0	2.8	62	4	14.3
	-	2.0	3.2	71	1	3.1
	-	4.0	5.4	120	1	1.9
	-	5.0	4.6	102	4	8.7
	+	1.0	3.4	81	3	8.8
	+	2.0	3.3	79	1	3.0
	+	4.0	5.0	119	2	4.0
	+	5.0	3.5	83	2	5.7

Table 67

IN VITRO ASSAYS WITH SACCHAROMYCES CEREVISIAE D3
TRICHLORFON

Compound	Metabolic Activation	Percent Concentration (w/v or v/v)	Survivors		Mitotic Recombinants	
			Cells per ml (x 10^{-7})	Percent	Per ml (x 10^{-3})	Per 10^5 Survivors
		EXPERIMENT 1				
Negative control	-		6.5	100	4	6.2
	+		6.4	100	2	3.1
Positive control	-	0.04	6.2	95	1235	1992
1,2,3,4-Diepoxybutane	+	0.04	5.8	91	835	1440
Trichlorfon	-	0.1	7.2	111	6	8.3
	-	0.5	6.4	98	25	39.1
	-	1.0	6.6	102	46	69.7
	-	5.0	0.3	5	T*	
	+	0.1	6.3	98	3	4.8
	+	0.5	5.0	78	9	18.0
	+	1.0	5.6	88	14	25.0
	+	5.0	4.0	63	197	493
		EXPERIMENT 2				
Negative control	-		6.8	100	7	10.3
	+		5.7	100	5	8.8
Positive control 1,2,3,4-Diepoxybutane	-	0.04	3.2	47	687	2147
Trichlorfon	-	1.0	6.7	99	28	41.8
	-	2.0	6.6	97	61	92.4
	-	4.0	5.1	75	171	335
	-	5.0	4.5	66	199	442
	+	1.0	6.4	112	60	93.8
	+	2.0	7.1	124	72	101
	+	4.0	7.2	126	153	212
	+	5.0	4.8	84	237	494

---

* T , toxic.

Table 68

UNSCHEDULED DNA SYNTHESIS ASSAY OF DEMETON
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Demeton (µg/ml)						4NQO (M)
Sample	0*	0.1	1.0	10	100	1000†	10^{-5}
1	67	32	33	53	244	--‡	4367
2	58	42	37	50	159	100	3593
3	--‡	42	67	40	294	95	2267
4	78	55	35	60	206	95	3039
5	71	50	45	68	211	74	2960
6	57	67	48	73	173	71	4142
Mean	66	48	44	57	214	87	3395
SD	9	12	13	12	49	13	791
SE	4	5	5	5	20	6	323

---

[*] Negative control and compound solvent, 0.5% EtOH.

[†] Precipitate observed at 1000 µg/ml.

[‡] Sample lost.

Table 69

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF DEMETON
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Demeton (µg/ml)						4NQO (M)
Sample	0*	50	100	200†	400†	800†	10^{-5}
1	68	161	166	215	155	90	1360
2	72	176	166	231	153	72	1237
3	75	190	264	280	188	129	1564
4	53	175	183	202	156	76	1095
5	49	146	174	183	131	82	1630
6	69	150	206	179	141	69	1381
Mean	64	166	193	215	154	87	1378
SD	11	17	38	37	19	22	199
SE	5	7	15	15	8	9	81

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitates observed at 200, 400, and 800 µg/ml.

Table 70

UNSCHEDULED DNA SYNTHESIS ASSAY OF DEMETON WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Demeton (µg/ml)						DMN (M)
Sample	0[*]	0.1	1.0	10	100	1000[†]	5×10^{-2}
1	87	31	73	71	89	147	577
2	117	45	106	61	69	169	505
3	87	42	84	80	79	134	550
4	98	37	88	64	93	149	485
5	97	36	91	80	71	142	518
6	104	28	97	84	117	137	529
Mean	98	36	90	73	87	146	527
SD	11	6	12	10	18	13	33
SE	5	3	5	4	7	5	14

---

[*] Negative control and compound solvent, 0.5% EtOH.

[†] Precipitate observed at 1000 µg/ml.

Table 71

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF DEMETON WITH METABOLIC ACTIVATION (dpm/μg DNA)

	Concentration of Compounds Tested						
	Demeton (μg/ml)						DMN (M)
Sample	0*	250†	500†	1000†	2000†	4000†	5×10^{-2}
1	69	62	122	153	127	53	387
2	87	90	135	155	133	104	306
3	--‡	95	86	159	134	85	368
4	69	84	84	162	138	78	326
5	77	85	90	148	185	105	388
6	113	114	115	119	140	47	362
Mean	83	88	106	149	142	78	356
SD	18	17	21	16	21	25	34
SE	8	7	9	6	9	10	14

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitates observed at all concentrations.

[‡] Sample lost.

Table 72

UNSCHEDULED DNA SYNTHESIS ASSAY OF ACEPHATE
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Acephate (μg/ml)						4NQO (M)
Sample	0*	0.1	1.0	10	100	1000	10^{-5}
1	223	116	165	186	179	314	3618
2	243	123	173	233	230	332	3807
3	242	208	134	217	209	323	2923
4	259	162	121	181	177	290	3397
5	225	129	137	182	220	280	2796
6	226	132	150	208	254	216	2889
Mean	236	145	147	201	212	292	3238
SD	14	35	20	22	30	42	427
SE	6	14	8	9	12	17	174

---

*Negative control and compound solvent, 0.5% DMSO.

Table 73

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF ACEPHATE
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Acephate (µg/ml)						
Sample	0*	125	250	500	1000	2000	4NQO (M) 10^{-5}
1	155	103	125	152	152	192	2387
2	127	118	149	154	154	163	2579
3	159	127	150	186	149	224	2657
4	120	124	152	159	213	173	2173
5	124	146	154	137	231	247	2093
6	127	134	121	136	199	172	2239
Mean	135	126	142	154	183	195	2355
SD	17	15	15	18	36	33	227
SE	7	6	6	7	15	14	93

---

*Negative control and compound solvent, 0.5% DMSO.

Table 74

UNSCHEDULED DNA SYNTHESIS ASSAY OF ACEPHATE WITH METABOLIC ACTIVATION (dpm/µg DNA)

	Concentration of Compounds Tested						
	Acephate (µg/ml)						DMN (M)
Sample	0*	0.1	1.0	10	100	1000	5×10^{-2}
1	207	142	140	208	216	242	574
2	202	139	139	157	193	156	507
3	231	110	127	123	251	152	580
4	150	138	153	193	214	224	420
5	167	174	141	184	176	228	410
6	165	169	159	155	186	247	433
Mean	187	145	143	170	206	208	487
SD	31	23	11	31	27	43	78
SE	13	10	5	13	11	18	32

---

*Negative control and compound solvent, 0.5% DMSO.

Table 75

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF ACEPHATE WITH METABOLIC ACTIVATION (dpm/μg DNA)

	Concentration of Compounds Tested						
	Acephate (μg/ml)						DMN (M)
Sample	0*	250	500	1000	2000	4000	5×10^{-2}
1	--†	65	61	75	69	89	486
2	106	70	50	87	70	75	299
3	88	75	55	77	71	64	252
4	59	83	62	81	70	69	256
5	83	80	83	61	89	81	242
6	69	93	49	73	70	64	414
Mean	81	78	60	76	73	74	325
SD	18	10	13	9	8	10	101
SE	8	4	5	4	3	4	41

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Sample lost.

Table 76

UNSCHEDULED DNA SYNTHESIS ASSAY OF DISULFOTON
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Disulfoton (μg/ml)						4NQO (M)
Sample	0[*]	0.1	1.0	10	100[†]	1000[†]	10^{-5}
1	67	40	33	23	64	92	4367
2	58	32	27	31	43	76	3593
3	--[‡]	40	38	43	59	111	2267
4	78	37	58	32	73	106	3039
5	71	26	30	36	68	109	2960
6	57	27	51	31	65	107	4142
Mean	66	34	39	33	62	100	3395
SD	9	6	12	7	11	14	791
SE	4	3	5	3	4	6	323

---

[*] Negative control and compound solvent, 0.5% EtOH.

[†] Precipitates observed at 100 and 1000 μg/ml.

[‡] Sample lost.

Table 77

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF DISULFOTON
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Disulfoton (µg/ml)						4NQO (M)
Sample	0*	250†	500†	1000†	2000†	4000†	10^{-5}
1	162	173	222	272	361	271	2293
2	158	142	200	294	258	335	2200
3	132	149	180	281	249	267	2604
4	109	163	214	209	267	272	2681
5	202	179	188	223	305	291	2066
6	160	202	218	237	397	248	2316
Mean	154	168	203	253	302	281	2360
SD	31	22	17	35	53	30	237
SE	13	9	7	14	22	12	97

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at all concentrations.

Table 78

UNSCHEDULED DNA SYNTHESIS ASSAY OF DISULFOTON WITH METABOLIC ACTIVATION (dpm/μg DNA)

	Concentration of Compounds Tested						
	Disulfoton (μg/ml)						DMN (M)
Sample	0*	0.1	1.0	10	100†	1000†	5×10^{-2}
1	223	188	163	146	211	163	1163
2	191	190	163	152	224	159	969
3	209	208	175	202	202	214	1023
4	204	209	77	164	173	202	956
5	181	218	165	165	220	227	1019
6	194	166	153	206	207	190	1008
Mean	200	197	149	172	206	193	1023
SD	15	19	36	25	18	27	74
SE	6	8	15	10	7	11	30

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitates observed at 100 and 1000 μg/ml.

Table 79

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF DISULFOTON WITH METABOLIC ACTIVATION (dpm/µg DNA)

	Concentration of Compounds Tested						
	Disulfoton (µg/ml)						DMN (M)
Sample	0*	250†	500†	1000†	2000†	4000†	5×10^{-2}
1	--‡	122	127	113	116	127	383
2	187	171	118	121	110	111	242
3	164	119	110	62	86	108	263
4	137	118	169	150	106	121	289
5	144	180	183	110	116	93	297
6	143	124	165	137	111	115	372
Mean	155	139	146	115	108	112	308
SD	21	29	31	30	11	12	58
SE	9	12	13	12	5	5	24

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at all concentrations.

‡Sample lost.

Table 80

UNSCHEDULED DNA SYNTHESIS ASSAY OF TRICHLORFON
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Trichlorfon (µg/ml)						4NQO (M)
Sample	0*	0.1	1.0	10	100	1000	10^{-5}
1	36	36	39	32	42	87	2076
2	33	42	32	31	26	83	1748
3	54	40	26	38	29	70	2349
4	34	26	23	37	50	56	2168
5	38	37	42	36	46	64	1771
6	31	47	28	55	34	--†	1814
Mean	37	38	32	38	38	72	1988
SD	8	7	8	9	10	13	247
SE	3	3	3	3	4	6	101

---

[*] Negative control and compound solvent, 0.5% EtOH.

[†] Sample lost.

Table 81

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF TRICHLORFON
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Trichlorfon (µg/ml)						4NQO (M)
Sample	0*	125	250	500	1000	2000	10^{-5}
1	155	162	188	210	230	341	2387
2	127	162	172	167	208	214	2579
3	159	162	194	237	178	232	2657
4	120	177	232	--†	224	226	2173
5	124	169	186	170	182	258	2093
6	127	221	224	208	211	229	2239
Mean	135	175	199	198	205	250	2355
SD	17	23	23	30	22	47	227
SE	7	9	9	13	9	19	93

---

*Negative control and compound solvent, 0.5% DMSO.

†Sample lost.

Table 82

UNSCHEDULED DNA SYNTHESIS ASSAY OF TRICHLORFON WITH METABOLIC ACTIVATION (dpm/µg DNA)

	Concentration of Compounds Tested						
	Trichlorfon (µg/ml)						DMN (M)
Sample	0*	0.1	1.0	10	100	1000	5×10^{-2}
1	113	65	76	107	48	112	400
2	102	98	95	102	115	168	397
3	141	105	96	130	163	132	--†
4	158	55	74	107	129	159	529
5	218	89	74	71	100	131	645
6	136	84	117	98	150	158	448
Mean	145	83	89	102	117	143	484
SD	41	19	17	19	41	22	105
SE	17	8	7	8	17	9	50

---

*Negative control and compound solvent, 0.5% EtOH.

†Sample lost.

Table 83

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF TRICHLORFON WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Trichlorfon (μg/ml)						DMN (M)
Sample	0*	250	500	1000	2000	4000	5×10^{-2}
1	--†	48	73	70	84	--†	486
2	106	71	76	91	105	105	299
3	88	40	61	64	80	83	252
4	59	59	57	86	113	63	256
5	83	72	52	97	72	73	242
6	69	75	82	76	70	79	414
Mean	81	61	67	81	87	81	325
SD	18	14	12	13	18	16	101
SE	8	6	5	5	8	7	41

---

*Negative control and compound solvent, 0.5% DMSO.

†Sample lost.

Table 84

UNSCHEDULED DNA SYNTHESIS ASSAY OF DICAMBA
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Dicamba (µg/ml)						4NQO (M)
Sample	0*	0.1	1.0	10	100	1000†	10^{-5}
1	152	125	142	122	136	73	2671
2	148	109	151	134	146	71	2538
3	140	125	75	162	177	119	2660
4	99	110	117	154	105	101	2443
5	138	111	121	160	135	115	2652
6	167	114	115	125	121	97	1455
Mean	141	116	120	143	137	96	2458
SD	23	7	27	18	25	21	497
SE	9	3	11	7	10	8	203

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 1000 µg/ml.

Table 85

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF DICAMBA
(dpm/μg DNA)

Sample	Concentration of Compounds Tested Dicamba (μg/ml) 0*	125	250	500	1000	2000†	4NQO (M) 10^{-5}
1	61	59	52	59	58	30	1760
2	70	72	62	64	49	41	1269
3	70	56	77	80	67	28	533
4	86	60	64	72	50	44	1878
5	55	70	52	57	67	35	1907
6	59	60	56	66	63	43	1929
Mean	67	63	60	66	59	37	1546
SD	11	6	9	8	8	7	554
SE	4	3	4	3	3	3	226

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitate observed at 2000 μg/ml.

Table 86

UNSCHEDULED DNA SYNTHESIS ASSAY OF DICAMBA WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Dicamba (µg/ml)						DMN (M)
Sample	0*	0.1	1.0	10	100	1000†	5 x 10^{-2}
1	101	81	91	98	105	133	468
2	76	89	82	94	107	116	639
3	54	81	101	102	111	105	842
4	67	79	83	90	82	128	576
5	89	76	66	99	124	97	739
6	38	95	100	113	83	164	819
Mean	71	84	87	99	102	124	681
SD	23	7	13	8	17	24	146
SE	9	3	5	3	7	10	60

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 1000 µg/ml.

Table 87

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF DICAMBA WITH METABOLIC ACTIVATION (dpm/μg DNA)

	Concentration of Compounds Tested						
	Dicamba (μg/ml)						DMN (M)
Sample	0*	37	111	333	1000†	3000†	5 x 10^{-2}
1	83	45	56	52	67	62	269
2	153	59	69	66	53	60	224
3	64	74	73	65	72	60	229
4	93	47	60	75	61	63	220
5	84	106	93	63	98	53	235
6	147	74	91	67	118	76	247
Mean	104	68	74	65	78	62	237
SD	37	23	16	7	25	8	18
SE	15	9	6	3	10	3	7

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 1000 and 3000 μg/ml.

Table 88

THIRD UNSCHEDULED DNA SYNTHESIS ASSAY OF DICAMBA WITH METABOLIC ACTIVATION
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Dicamba (μg/ml)						DMN (M)
Sample	0*	37	111	333	1000†	3000†	5 x 10^{-2}
1	75	74	111	85	66	86	567
2	81	80	80	78	89	65	596
3	90	42	82	90	55	148	492
4	41	71	41	85	61	87	506
5	72	74	46	95	114	85	472
6	57	81	123	84	66	136	437
Mean	69	70	81	86	75	101	512
SD	18	15	33	6	22	33	59
SE	7	6	13	2	9	14	24

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 1000 and 3000 μg/ml.

Table 89

UNSCHEDULED DNA SYNTHESIS ASSAY OF FENSULFOTHION
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Fensulfothion (µg/ml)						4NQO (M)
Sample	0[*]	0.1	1.0	10	100	1000[†]	10^{-5}
1	--[‡]	63	38	28	12	4	1134
2	68	--[‡]	23	27	4	0	1101
3	54	38	40	47	13	5	1076
4	53	43	35	26	21	11	830
5	45	49	34	33	14	15	1190
6	47	36	34	29	13	2	976
Mean	53	46	34	32	13	6	1051
SD	9	11	6	8	5	6	129
SE	4	5	2	3	2	2	53

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitate observed at 1000 µg/ml.

[‡] Sample lost.

Table 90

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF FENSULFOTHION (dpm/μg DNA)

	Concentration of Compounds Tested						
	Fensulfothion (μg/ml)						4NQO (M)
Sample	0*	6.25	1.25	25	50	100	10^{-5}
1	61	42	58	36	30	18	1760
2	70	60	49	36	30	27	1269
3	70	55	52	53	35	22	533
4	86	56	52	58	36	23	1878
5	55	57	48	40	30	17	1907
6	59	52	49	47	27	30	1929
Mean	67	54	51	45	31	23	1546
SD	11	6	4	9	3	5	554
SE	4	2	2	4	1	2	226

---

*Negative control and compound solvent, 0.5% DMSO.

Table 91

UNSCHEDULED DNA SYNTHESIS ASSAY OF FENSULFOTHION WITH METABOLIC ACTIVATION (dpm/μg DNA)

	Concentration of Compounds Tested						
	Fensulfothion (μg/ml)						DMN (M)
Sample	0*	0.1	1.0	10	100	1000†	5×10^{-2}
1	101	79	124	103	148	107	468
2	76	111	130	163	118	95	639
3	54	114	139	169	118	142	842
4	67	103	125	160	155	142	576
5	89	131	174	126	180	131	739
6	38	130	137	128	128	118	819
Mean	71	112	138	142	141	123	681
SD	23	19	19	27	24	19	146
SE	9	8	8	11	10	8	60

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 1000 μg/ml.

Table 92

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF FENSULFOTHION WITH METABOLIC ACTIVATION

(dpm/µg DNA)

	Concentration of Compounds Tested						
	Fensulfothion (µg/ml)						DMN (M)
Sample	0*	12	37	111	333	1000	5 x 10^{-2}
1	83	70	64	37	59	31	269
2	153	70	64	59	48	57	224
3	64	83	79	52	63	54	229
4	93	51	61	55	40	43	220
5	84	78	83	49	52	43	235
6	147	36	50	60	52	47	247
Mean	104	65	67	52	52	46	237
SD	37	18	12	9	8	9	18
SE	15	7	5	4	3	4	7

---

[*] Negative control and compound solvent, 0.5% DMSO.

Table 93

UNSCHEDULED DNA SYNTHESIS ASSAY OF ENDRIN
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Endrin (μg/ml)						4NQO (M)
Sample	0*	0.1	1.0	10	100†	1000†	10^{-5}
1	149	78	103	95	116	119	1271
2	103	80	89	94	87	100	1288
3	111	98	74	92	92	73	1421
4	109	75	89	95	94	106	1271
5	99	56	84	99	82	108	1363
6	106	72	103	110	118	121	1446
Mean	113	76	90	97	98	105	1343
SD	18	14	11	7	15	17	78
SE	8	6	5	3	6	7	32

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 100 and 1000 μg/ml.

Table 94

UNSCHEDULED DNA SYNTHESIS ASSAY OF ENDRIN WITH METABOLIC ACTIVATION (dpm/μg DNA)

	Concentration of Compounds Tested						
	Endrin (μg/ml)						DMN (M)
Sample	0[*]	0.1	1.0	10	100[†]	1000[†]	5×10^{-2}
1	162	76	103	151	156	185	690
2	138	97	116	148	138	213	360
3	122	109	106	115	181	206	448
4	182	110	53	129	124	176	348
5	106	99	90	111	165	289	388
6	166	121	108	143	159	134	--[‡]
Mean	143	102	96	133	154	201	447
SD	28	15	23	17	20	52	141
SE	11	6	9	7	8	21	63

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitates observed at 100 and 1000 μg/ml.

[‡] Sample lost.

Table 95

REPEAT UNSCHEDULED DNA SYNTHESIS ASSAY OF ENDRIN WITH METABOLIC ACTIVATION
(dpm/μg DNA)

Sample	Concentration of Compounds Tested						
	Endrin (μg/ml)						DMN (M)
	0[*]	188[†]	375[†]	750[†]	1500[†]	3000[†]	5×10^{-2}
1	--[‡]	93	134	114	151	187	383
2	187	141	151	131	147	135	242
3	164	141	133	132	165	160	263
4	137	173	--[‡]	190	130	163	289
5	144	156	106	118	148	178	297
6	143	144	122	136	194	219	372
Mean	155	141	129	137	156	174	308
SD	21	27	17	27	22	29	58
SE	9	11	7	11	9	11	24

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitates observed at all concentrations.

[‡] Sample lost.

Table 96

THIRD UNSCHEDULED DNA SYNTHESIS ASSAY OF ENDRIN WITH METABOLIC ACTIVATION (dpm/μg DNA)

	Concentration of Compounds Tested						
	Endrin (μg/ml)						DMN (M)
Sample	0*	37†	111†	333†	1000†	3000†	5 x 10^{-2}
1	72	70	72	67	72	62	348
2	79	73	64	75	80	65	363
3	87	84	63	69	77	62	352
4	77	101	93	63	82	80	377
5	77	66	79	64	73	74	297
6	63	62	65	76	71	59	183
Mean	76	76	73	69	76	67	320
SD	8	14	12	5	4	8	72
SE	3	6	5	2	2	3	30

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at all concentrations.

Table 97

UNSCHEDULED DNA SYNTHESIS ASSAY OF ASPON
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Aspon (μg/ml)						4NQO (M)
Sample	0[*]	0.1	1.0	10	100[†]	1000[†]	10^{-5}
1	223	169	163	221	286	234	3618
2	243	190	183	234	248	228	3807
3	242	175	141	266	305	293	2923
4	259	163	145	243	194	228	3397
5	225	187	165	369	229	269	2796
6	226	--[‡]	184	208	263	198	2889
Mean	236	177	163	257	254	242	3238
SD	14	11	18	58	40	34	427
SE	6	5	7	24	16	14	174

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitates observed at 100 and 1000 μg/ml.

[‡] Sample lost.

Table 98

UNSCHEDULED DNA SYNTHESIS ASSAY OF ASPON WITH METABOLIC ACTIVATION (dpm/μg DNA)

	Concentration of Compounds Tested						
	Aspon (μg/ml)						DMN (M)
Sample	0[*]	0.1	1.0	10	100[†]	1000[†]	5×10^{-2}
1	223	184	199	170	175	239	1163
2	191	173	146	165	211	197	969
3	209	160	174	222	168	219	1023
4	204	120	130	--[‡]	159	185	956
5	181	92	150	--[‡]	198	210	1019
6	194	116	124	154	176	160	1008
Mean	200	132	154	178	181	202	1023
SD	15	30	28	30	19	28	74
SE	6	12	12	15	8	11	30

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitates observed at 100 and 1000 μg/ml.

[‡] Sample lost.

Table 99

UNSCHEDULED DNA SYNTHESIS ASSAY OF CARBOFURAN
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Carbofuran (μg/ml)						4NQO (M)
Sample	0*	0.1	1.0	10	100	1000†	10^{-5}
1	73	63	60	42	64	55	2694
2	100	65	58	56	74	54	2325
3	77	61	72	52	68	51	2592
4	112	39	48	82	64	83	2404
5	77	59	55	82	86	69	2223
6	74	47	63	59	76	49	2292
Mean	85	56	59	62	72	60	2422
SD	17	10	8	16	9	13	184
SE	7	4	3	7	3	5	74

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitate observed at 1000 μg/ml.

Table 100

UNSCHEDULED DNA SYNTHESIS ASSAY OF CARBOFURAN WITH METABOLIC ACTIVATION (dpm/µg DNA)

	Concentration of Compounds Tested						
	Carbofuran (µg/ml)						DMN (M)
Sample	0[*]	0.1	1.0	10	100	1000[†]	5×10^{-2}
1	87	67	82	89	81	189	577
2	117	116	86	53	106	118	505
3	87	74	73	62	71	84	550
4	98	121	55	64	70	93	485
5	97	88	63	53	86	93	518
6	104	96	76	71	84	99	529
Mean	98	94	73	67	83	113	527
SD	11	22	12	17	13	39	33
SE	5	9	5	7	5	16	14

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitate observed at 1000 µg/ml.

Table 101

UNSCHEDULED DNA SYNTHESIS ASSAY OF CROTOXYPHOS
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Crotoxyphos (µg/ml)						4NQO (M)
Sample	0[*]	0.1	1.0	10	100	1000[†]	10^{-5}
1	73	80	72	149	42	15	2694
2	100	99	109	121	43	10	2325
3	77	93	100	62	33	18	2592
4	112	128	146	88	40	12	2404
5	77	109	141	154	54	11	2223
6	74	126	105	100	44	11	2292
Mean	85	106	112	116	43	13	2422
SD	17	19	28	31	7	3	184
SE	7	8	11	13	3	1	75

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitate observed at 1000 µg/ml.

Table 102

UNSCHEDULED DNA SYNTHESIS ASSAY OF CROTOXYPHOS WITH METABOLIC ACTIVATION (dpm/µg DNA)

	Concentration of Compounds Tested						
	Crotoxyphos (µg/ml)						DMN (M)
Sample	0[*]	0.1	1.0	10	100	1000[†]	5×10^{-2}
1	162	144	65	117	101	122	690
2	138	165	115	77	83	139	360
3	122	221	93	111	111	119	448
4	182	180	63	124	143	115	348
5	106	176	222	76	102	161	388
6	166	158	84	90	99	139	--[‡]
Mean	143	174	82	99	106	133	447
SD	28	26	55	21	20	17	141
SE	11	11	22	8	8	7	63

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitate observed at 1000 µg/ml.

[‡] Sample lost.

Table 103

UNSCHEDULED DNA SYNTHESIS ASSAY OF 2,4-D ACID
(dpm/μg DNA)

	Concentration of Compounds Tested						
	2,4-D (μg/ml)						4NQO (M)
Sample	0[*]	0.1	1.0	10	100	1000	10^{-5}
1	153	115	98	149	149	206	3589
2	164	107	132	159	161	197	3320
3	204	111	131	150	143	196	2671
4	161	119	119	198	138	175	2769
5	182	81	101	157	151	173	2494
6	195	--[†]	169	163	149	173	2644
Mean	177	107	125	163	148	186	2914
SD	20	15	25	18	8	15	436
SE	8	7	11	7	3	6	178

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Sample lost.

Table 104

UNSCHEDULED DNA SYNTHESIS ASSAY OF 2,4-D ACID WITH METABOLIC ACTIVATION

(dpm/µg DNA)

	Concentration of Compounds Tested						
	2,4-D (µg/ml)						DMN (M)
Sample	0*	0.1	1.0	10	100	1000	5 x 10^{-2}
1	165	162	131	128	146	81	334
2	125	143	176	122	123	126	328
3	134	130	81	127	143	128	291
4	142	165	141	138	138	137	330
5	159	128	112	136	160	139	304
6	142	141	147	121	117	107	264
Mean	144	145	131	128	138	120	308
SD	15	16	32	7	16	22	27
SD	6	6	13	3	7	9	11

---

*Negative control and compound solvent, 0.5% DMSO.

Table 105

UNSCHEDULED DNA SYNTHESIS ASSAY OF 2,4-DB ACID
(dpm/μg DNA)

	Concentration of Compounds Tested						
	2,4-DB (μg/ml)						4NQO (M)
Sample	0*	0.1	1.0	10	100	1000	10^{-5}
1	153	74	107	197	123	118	3589
2	164	118	129	109	168	67	3320
3	204	85	111	107	136	143	2671
4	161	109	109	134	150	147	2769
5	182	117	100	116	191	66	2494
6	195	79	98	91	--†	94	2644
Mean	177	97	109	126	154	106	2914
SD	20	20	11	38	27	36	436
SE	8	8	5	15	12	15	178

---

*Negative control and compound solvent, 0.5% DMSO.

†Sample lost.

Table 106

UNSCHEDULED DNA SYNTHESIS ASSAY OF 2,4-DB ACID WITH METABOLIC ACTIVATION

(dpm/μg DNA)

	Concentration of Compounds Tested						
	2,4-DB (μg/ml)						DMN (M)
Sample	0*	0.1	1.0	10	100	1000	5×10^{-2}
1	165	117	122	129	141	120	334
2	125	132	117	181	106	115	328
3	134	154	122	134	119	111	291
4	142	119	126	133	146	115	330
5	159	121	116	143	171	146	304
6	142	176	133	164	145	119	264
Mean	144	137	123	147	138	121	308
SD	15	24	6	21	23	13	27
SE	6	10	3	8	9	5	11

---

*Negative control and compound solvent, 0.5% DMSO.

Table 107

UNSCHEDULED DNA SYNTHESIS ASSAY OF DIAZINON
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Diazinon (μg/ml)						4NQO (M)
Sample	0[*]	0.1	1.0	10	100[†]	1000[†]	10^{-5}
1	36	44	36	61	20	0	2076
2	33	57	43	44	14	0	1748
3	54	65	40	31	12	0	2349
4	34	51	22	25	23	26	2168
5	38	43	65	25	34	0	1771
6	31	49	52	22	40	3	1814
Mean	37	51	43	35	24	5	1988
SD	8	8	15	15	11	10	247
SE	3	3	6	6	4	4	101

---

[*] Negative control and compound solvent, 0.5% EtOH.

[†] Precipitates observed at 100 and 1000 μg/ml

Table 108

UNSCHEDULED DNA SYNTHESIS ASSAY OF DIAZINON WITH METABOLIC ACTIVATION
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Diazinon (µg/ml)						DMN (M)
Sample	0*	0.1	1.0	10	100†	1000†	5×10^{-2}
1	94	77	87	102	95	113	327
2	107	82	78	100	85	122	399
3	132	72	81	93	100	75	343
4	118	71	99	52	98	77	429
5	153	72	82	120	80	114	372
6	120	77	107	98	116	90	455
Mean	121	75	89	94	96	98	387
SD	20	4	11	23	13	20	50
SE	8	2	5	9	5	8	20

---

*Negative control and compound solvent, 0.5% EtOH.

†Precipitates observed at 100 and 1000 µg/ml.

Table 109

UNSCHEDULED DNA SYNTHESIS ASSAY OF FONOFOS
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Fonofos (μg/ml)						4NQO (M)
Sample	0*	0.1	1.0	10	100†	1000†	10^{-5}
1	61	43	46	52	55	67	2022
2	49	44	50	59	77	75	1046
3	53	66	55	66	76	92	2460
4	49	66	67	92	85	38	2090
5	42	35	49	65	49	54	2177
6	43	46	33	63	71	27	2179
Mean	49	50	50	66	69	59	1995
SD	7	13	11	14	14	24	489
SE	3	5	5	6	6	2	199

---

[*] Negative control and compound solvent 0.5% DMSO.

[†] Precipitate observed at 100 and 1000 μg/ml.

Table 110

UNSCHEDULED DNA SYNTHESIS ASSAY OF FONOFOS WITH METABOLIC ACTIVATION (dpm/μg DNA)

	Concentration of Compounds Tested						
	Fonofos (μg/ml)						DMN (M)
Sample	0*	0.1	1.0	10	100†	1000†	5×10^{-2}
1	94	115	128	70	96	125	327
2	107	111	112	110	131	125	399
3	132	95	153	110	143	149	343
4	118	131	85	131	170	186	429
5	153	130	114	95	132	121	372
6	120	134	135	110	130	151	455
Mean	121	119	121	104	134	143	387
SD	20	15	23	20	24	25	50
SE	8	6	10	8	10	10	20

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 100 and 1000 μg/ml.

Table 111

UNSCHEDULED DNA SYNTHESIS ASSAY OF ETHION
(dpm/µg DNA)

	Concentration of Compounds Tested						
	Ethion (µg/ml)						4NQO (M)
Sample	0*	0.1	1.0	10	100†	1000†	10^{-5}
1	163	196	171	160	117	119	3144
2	173	100	186	220	136	248	3221
3	173	128	188	102	159	147	3781
4	196	101	150	141	192	124	3556
5	188	255	165	186	171	173	3374
6	219	--‡	143	166	182	151	--‡
Mean	186	156	167	162	160	160	3415
SD	20	68	18	40	28	47	258
SE	8	30	8	16	11	19	116

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitates observed at 100 and 1000 µg/ml.

‡Sample lost.

Table 112

UNSCHEDULED DNA SYNTHESIS ASSAY OF ETHION WITH METABOLIC ACTIVATION (dpm/µg DNA)

	Concentration of Compounds Tested						
	Ethion (µg/ml)						DMN (M)
Sample	0[*]	0.1	1.0	10	100[†]	1000[†]	5×10^{-2}
1	142	70	98	92	91	89	374
2	109	82	80	83	95	72	366
3	67	110	108	90	105	105	426
4	69	104	112	87	105	100	397
5	93	117	90	102	113	109	435
6	113	103	90	114	119	109	449
Mean	99	96	96	95	105	97	408
SD	29	17	12	12	11	14	34
SE	12	7	5	5	4	6	14

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitates observed at 100 and 1000 µg/ml.

Table 113

UNSCHEDULED DNA SYNTHESIS ASSAY OF METHOXYCHLOR
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Methoxychlor (μg/ml)						4NQO (M)
Sample	0*	0.1	1.0	10	100†	1000†	10^{-5}
1	149	75	84	86	86	102	1271
2	103	78	73	84	92	123	1288
3	111	88	104	77	83	87	1421
4	109	80	74	85	76	113	1271
5	99	87	72	100	119	106	1363
6	106	70	68	163	83	107	1446
Mean	113	79	79	99	90	106	1343
SD	18	8	13	32	15	12	78
SE	8	3	6	13	6	5	32

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitates observed at 100 and 1000 μg/ml.

Table 114

UNSCHEDULED DNA SYNTHESIS ASSAY OF METHOXYCHLOR WITH METABOLIC ACTIVATION (dpm/µg DNA)

	Concentration of Compounds Tested						
	Methoxychlor (µg/ml)						DMN (M)
Sample	0[*]	0.1	1.0	10	100[†]	1000[†]	5×10^{-2}
1	98	92	107	80	142	129	595
2	171	128	85	103	89	99	635
3	143	106	86	107	101	104	508
4	119	88	64	101	104	104	487
5	160	94	82	114	132	99	670
6	126	76	54	--[‡]	102	89	661
Mean	136	97	80	101	112	104	593
SD	27	18	19	13	20	13	78
SE	11	7	8	5	8	5	32

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitates observed at 100 and 1000 µg/ml.

[‡] Sample lost.

Table 115

UNSCHEDULED DNA SYNTHESIS ASSAY OF SIDURON
(dpm/μg DNA)

Sample	Concentration of Compounds Tested Siduron (μg/ml) 0*	 0.1	 1.0	 10	 100	 1000†	 4NQO (M) 10^{-5}
1	--‡	41	32	33	16	26	1134
2	68	32	38	34	15	19	1101
3	54	30	30	26	14	26	1076
4	53	29	24	25	17	29	830
5	45	39	28	37	21	22	1190
6	47	39	29	32	15	19	976
Mean	53	35	32	31	16	24	1051
SD	9	5	6	5	3	4	129
SE	4	2	2	2	1	1	53

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 1000 μg/ml.

‡Sample lost.

Table 116

UNSCHEDULED DNA SYNTHESIS ASSAY OF SIDURON WITH METABOLIC ACTIVATION (dpm/μg DNA)

	Concentration of Compounds Tested						
	Siduron (μg/ml)						DMN (M)
Sample	0*	0.1	1.0	10	100	1000†	5×10^{-2}
1	207	224	64	137	176	175	574
2	202	162	142	108	178	118	507
3	231	294	132	130	103	203	580
4	150	196	99	150	163	126	420
5	167	173	148	151	132	136	410
6	165	206	134	195	172	156	433
Mean	187	209	120	145	154	152	487
SD	31	47	32	29	30	32	78
SE	13	19	13	12	12	13	32

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 1000 μg/ml.

Table 117

UNSCHEDULED DNA SYNTHESIS ASSAY OF PROPANIL
(dpm/μg DNA)

	Concentration of Compounds Tested						
	Propanil (μg/ml)						4NQO (M)
Sample	0*	0.1	1.0	10	100	1000†	10^{-5}
1	152	104	120	117	24	30	2671
2	148	147	105	145	36	28	2538
3	140	79	136	117	51	23	2660
4	99	55	111	109	31	101	2773
5	138	52	112	105	39	99	2652
6	167	103	148	81	22	0	1455
Mean	141	89	122	112	34	47	2458
SD	23	36	17	21	11	43	497
SE	9	15	7	9	4	17	203

---

[*] Negative control and compound solvent, 0.5% DMSO.

[†] Precipitate observed at 1000 μg/ml.

Table 118

UNSCHEDULED DNA SYNTHESIS ASSAY OF PROPANIL WITH METABOLIC ACTIVATION (dpm/µg DNA)

	Concentration of Compounds Tested						
	Propanil (µg/ml)						DMN (M)
Sample	0*	0.1	1.0	10	100	1000†	5 x 10^{-2}
1	98	76	75	76	113	107	595
2	171	67	67	95	73	85	635
3	143	78	73	111	97	105	508
4	119	64	95	76	124	105	487
5	160	63	64	91	104	104	670
6	126	67	65	76	85	89	661
Mean	136	69	73	87	100	99	593
SD	27	6	12	14	19	10	78
SE	11	3	5	6	8	4	32

---

*Negative control and compound solvent, 0.5% DMSO.

†Precipitate observed at 1000 µg/ml.

REFERENCES

1. V. F. Simmon, A. D. Mitchell, and T. A. Jorgenson. Evaluation of select pesticides as chemical mutagens: *In vitro* and *in vivo* studies. Environmental Health Effects Research Series EPA-600/1-77-028, May 1977.
2. J. McCann, E. Choi, E. Yamasaki, and B. N. Ames. Detection of carcinogens as mutagens in the *Salmonella* microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. USA *72*, 5135-5139 (1975).
3. I. F. H. Purchase, E. Longstaff, J. Ashby, J. A. Styles, D. Anderson, P. A. Lafevre, and F. R. Westwood. Evaluation of six short term tests for detecting organic chemical carcinogens and recommendations for their use. Nature *264*, 624-527 (1976).
4. V. F. Simmon. *In vitro* assays for recombinogenic activity of chemical carcinogens and related compounds with *Saccharomyces cerevisiae* D3. J. Nat. Cancer Inst. *62* (in press) (1979).
5. B. N. Ames, E. G. Gurney, J. A. Miller, and H. Bartsch. Carcinogens as frameshift mutagens: Metabolites and derivatives of 2-acetylaminofluorene and other aromatic amine carcinogens. Proc. Nat. Acad. Sci. USA *69*, 3128-3132 (1972).
6. B. N. Ames, F. D. Lee, and W. E. Durston. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Nat. Acad. Sci. USA *70*, 782-786 (1973).
7. J. McCann, N. E. Spingarn, J. Kobori, and B. N. Ames. The detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. Proc. Nat. Acad. Sci. USA *72*, 979-983 (1975).
8. B. N. Ames, W. E. Durston, E. Yamasaki, and F. D. Lee. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci. USA *70*, 2281-2285 (1973).
9. L. D. Kier, E. Yamasaki, and B. N. Ames. Detection of mutagenic activity in cigarette smoke condensates. Proc. Nat. Acad. Sci. USA *71*, 4159-4163 (1974).
10. B. N. Ames, J. McCann, and E. Yamasaki. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutation Res. *31*, 347-364 (1975).
11. D. R. McCalla and D. Voutsinos. On mutagenicity of nitrofurans. Mutation Res. *26*, 3-16 (1974).
12. B. A. Bridges. Simple bacterial systems for detecting mutagenic agents. Lab. Pract. *21*, 413-419 (1972).

13. F. K. Zimmermann and R. Schwaier. A genetic effect of symmetric dimethylhydraine: Induction of mitotic recombination. Naturwissenschaften 54, 251 (1967).

14. D. J. Brusick and V. W. Mayer. New developments in mutagenicity screening techniques with yeast. Envir. Health Perspectives 6, 83-96 (1973).

15. E. E. Slater, M. D. Anderson, and H. S. Posenkranz. Rapid detection of mutagens and carcinogens. Cancer Res. 31, 970-973 (1971).

16. J. Gross and M. Gross. Genetic analysis of an E. coli strain with a mutation affecting DNA polymerase. Nature 224, 1166-1168 (1969).

17. T. Kada. Mutagenicity testing of chemicals in microbial systems. In New Methods in Environmental Chemistry and Toxicology. International Academic Printing Co., Totsuka, Tokyo, 1973.

18. R. H. C. San and H. F. Stich. DNA repair synthesis of cultured human cells as a rapid bioassay for chemical carcinogens. Int. J. Cancer 16, 284-291 (1975).

19. B. Djordjevic and L. Tolmach. Response of synchronized populations of HeLa cells to ultraviolet irradiation at selected stages of the generation cycle. Radiat. Res. 32, 327 (1967).

20. R. E. Rasmussen and R. B. Painter. Radiation-stimulated DNA synthesis in cultured mammalian cells. J. Cell Biol. 29, 11 (1966).

21. G. Schmidt and S. J. Thannhauser. A method for the determination of deoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. J. Biol. Chem. 161, 83-89 (1945).

22. G. M. Richards. Modification of the dipheylamine reaction giving increased sensitivity and simplicity in the estimation of DNA. Anal. Biochem. 57, 369-374 (1974).

23. M. R. Spiegel. Theory and Problems of Statistics. Schamm Publishing Co., New York, 1961, pp. 241-248.

24. W. C. Guenther. Analysis of Variance. Pretice-Hall, Inc., Englewood Cliffs, N. J., 1964, pp. 20-21, 31-43.

25. S. Siegel. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill Book Compnay, New York, 1956, pp. 184-194.

26. S. S. Epstein, E. Arnold, J. Andrea, W. Bass, and V. Bishop. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol. Appl. Pharmacol. 23, 288-325 (1972).

27. V. V. Brsheskii. Possibility of induction of mutations in Drosophila melanogaster with chlorophos. Med. Parasit. Parazil. Bolez. 42, 703-706 (1973).

28. IARC. 2,4-D and Esters. In IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man Vol. 15, IARC, Lyon, France, 1977, pp. 111-139.

29. Y. Shirasu. Significance of mutagenicity testing on pesticides. Environ. Qual. Sat. 4, 226-231 (1975).